

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Baltimore, et al.	Attorney Docket No.	APBI-P04-035
Serial No: To be assigned	Art Unit:	To be assigned
Filed: January 4, 2002	Examiner:	To be assigned
For: Nuclear Factors Associated with Transcriptional Regulation		

Assistant Commissioner for Patents
U.S. Patent and Trademark Office
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Please enter the following amendment:

In the specification:

Please replace the only complete paragraph under the heading Related Applications on page 1 with the following text:

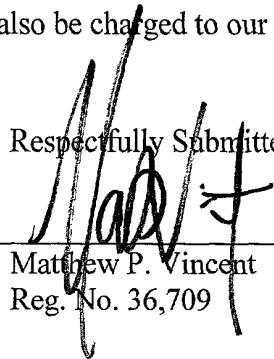
This application is a continuation of Serial No. 08/464,364, filed June 5, 1995, which is a divisional of Serial No. 08/418,266, filed April 6, 1995, which is a continuation of 07/791,898, filed November 13, 1991, which is a continuation-in-part of application of Serial No. 06/946,365 (WHI86-10), filed December 24, 1986, and of Serial No. 07/318,901 (WHI87-11A), filed March 3, 1989, and of Serial No. 07/162,680 (WHI87-11), filed March 1, 1988, and of Serial No. 07/341,436 (WHI89-02) filed April 21, 1989, and of Serial No. 06/817/441 (MIT-4167), filed January 9, 1986, and of Serial No. 07/155,207 (MIT-4167A), filed February 12, 1988, and of Serial No. 07/280,173 (MIT-4167AA), filed December 5, 1988. The contents of the ten referenced applications are incorporated herein by reference.

The replacement paragraph presented above incorporates changes as indicated by the marked-up version below.

This application is a continuation of Serial No. 08/464,364, filed June 5, 1995, which is a divisional of Serial No. 08/418,266, filed April 6, 1995, which is a continuation of 07/791,898, filed November 13, 1991, which is a continuation-in-part of application of Serial No. 06/946,365 (WHI86-10), filed December 24, 1986; and of Serial No. 07/318,901 (WHI87-11A), filed March 3, 1989; and of Serial No. 07/162,680 (WHI87-11), filed March 1, 1988; and of Serial No. 07/341,436 (WHI89-02) filed April 21, 1989; and of Serial No. 06/817/441 (MIT-4167), filed January 9, 1986; and of Serial No. 07/155,207 (MIT-4167A), filed February 12, 1988, and of Serial No. 07/280,173 (MIT-4167AA), filed December 5, 1988. The contents of the ~~seven~~ten referenced applications are incorporated herein by reference.

Although Applicant believes no fees are needed in connection with filing this Preliminary Amendment, should fees be due in connection with the filing of this Amendment, please charge the fees to our **Deposit Account No. 18-1945**. If a fee is required for an extension of time under 37 C.F.R. § 1.136 not accounted for above, such an extension is requested and the fee should also be charged to our Deposit account.

Respectfully Submitted,


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J1046 U.S. PTO
10/037415
01/04/02

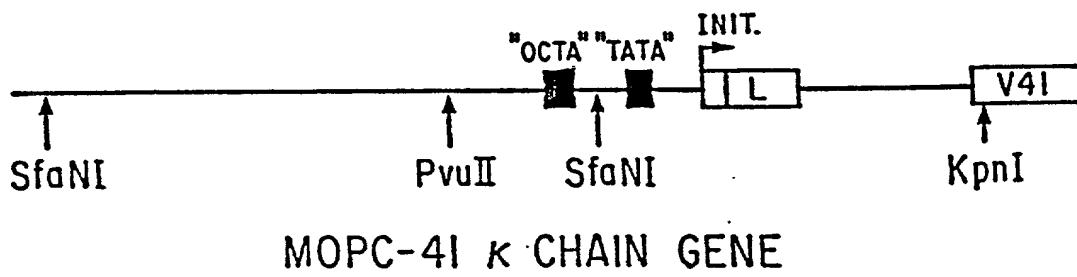


FIG. 1A

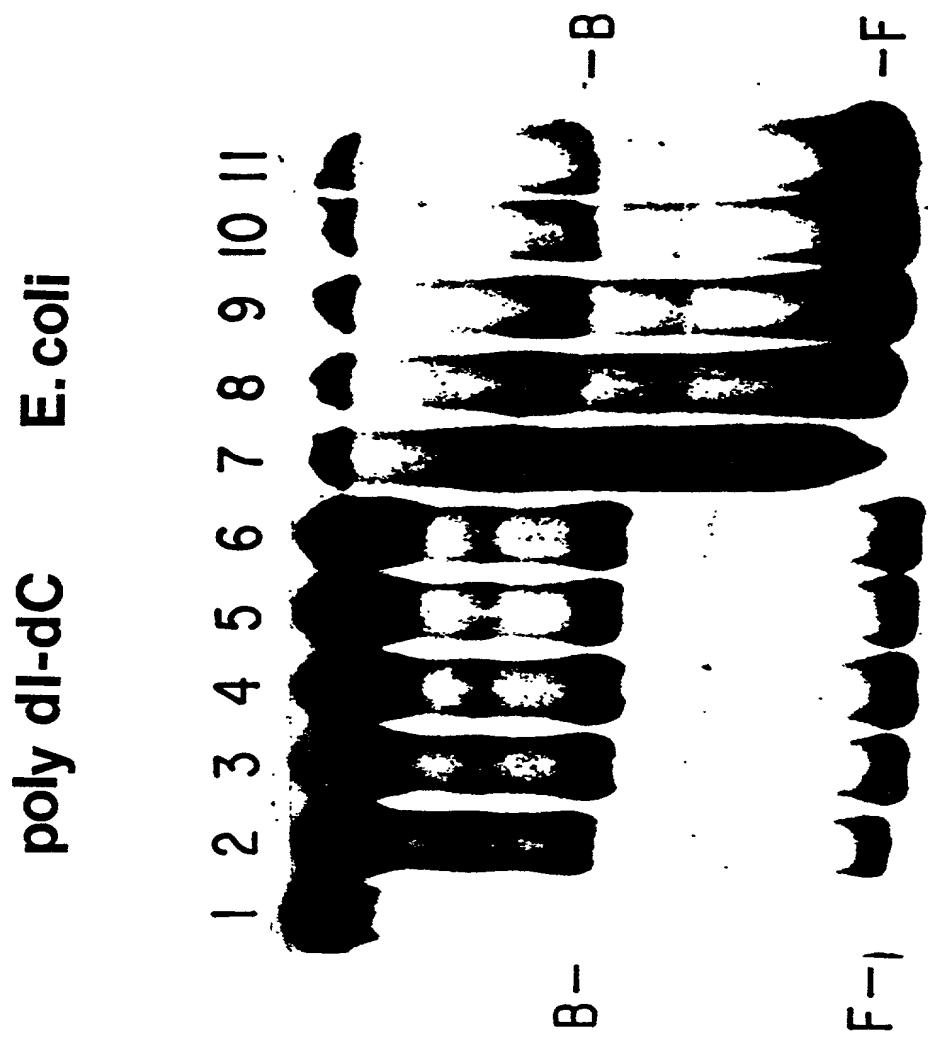


FIG. 1B

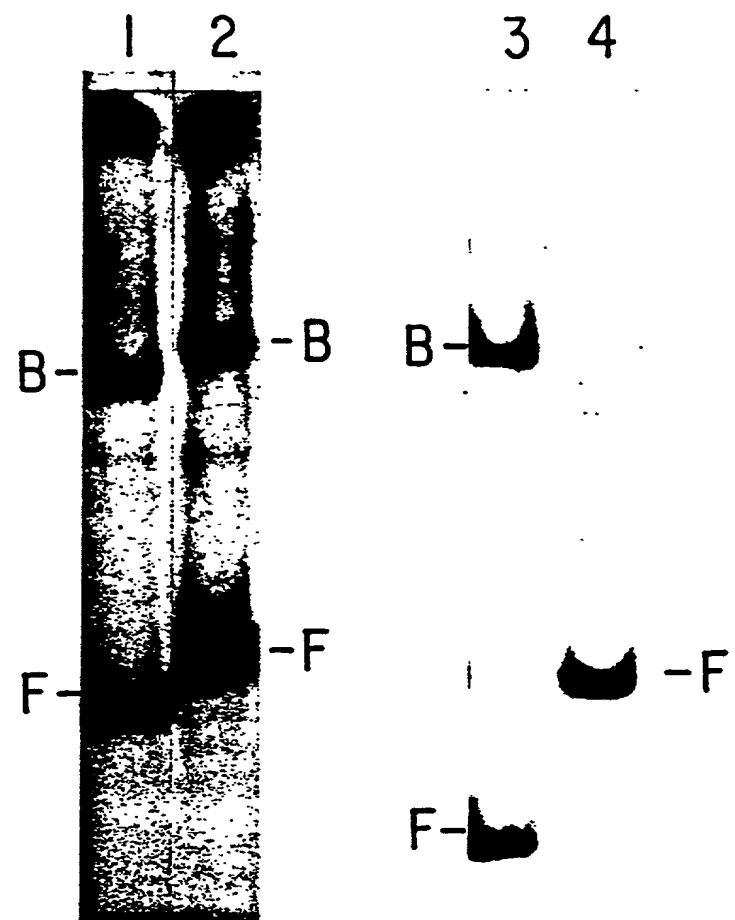


FIG.1C

pUC VL

1 2 3 4 5 6 7



FIG.2A

FIG.2B

1 2



HeLa

FIG.3

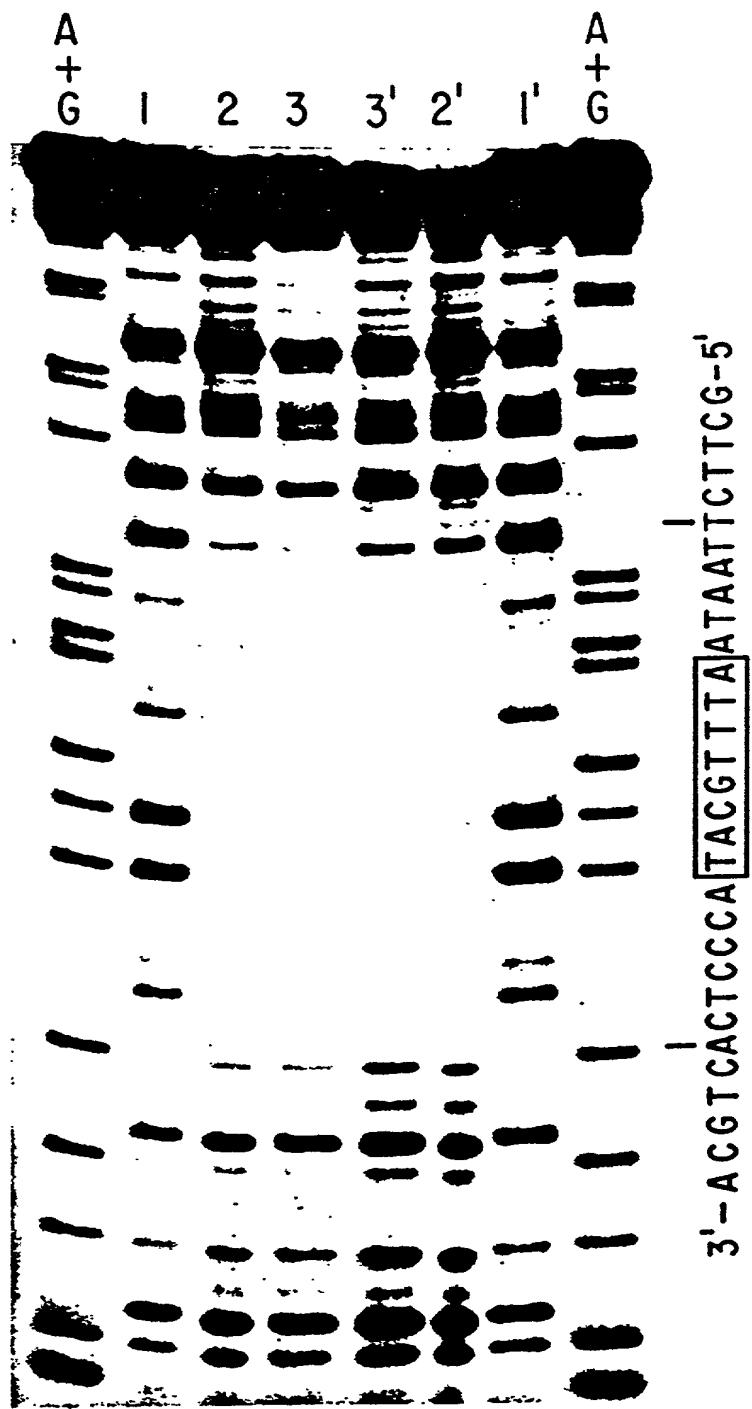
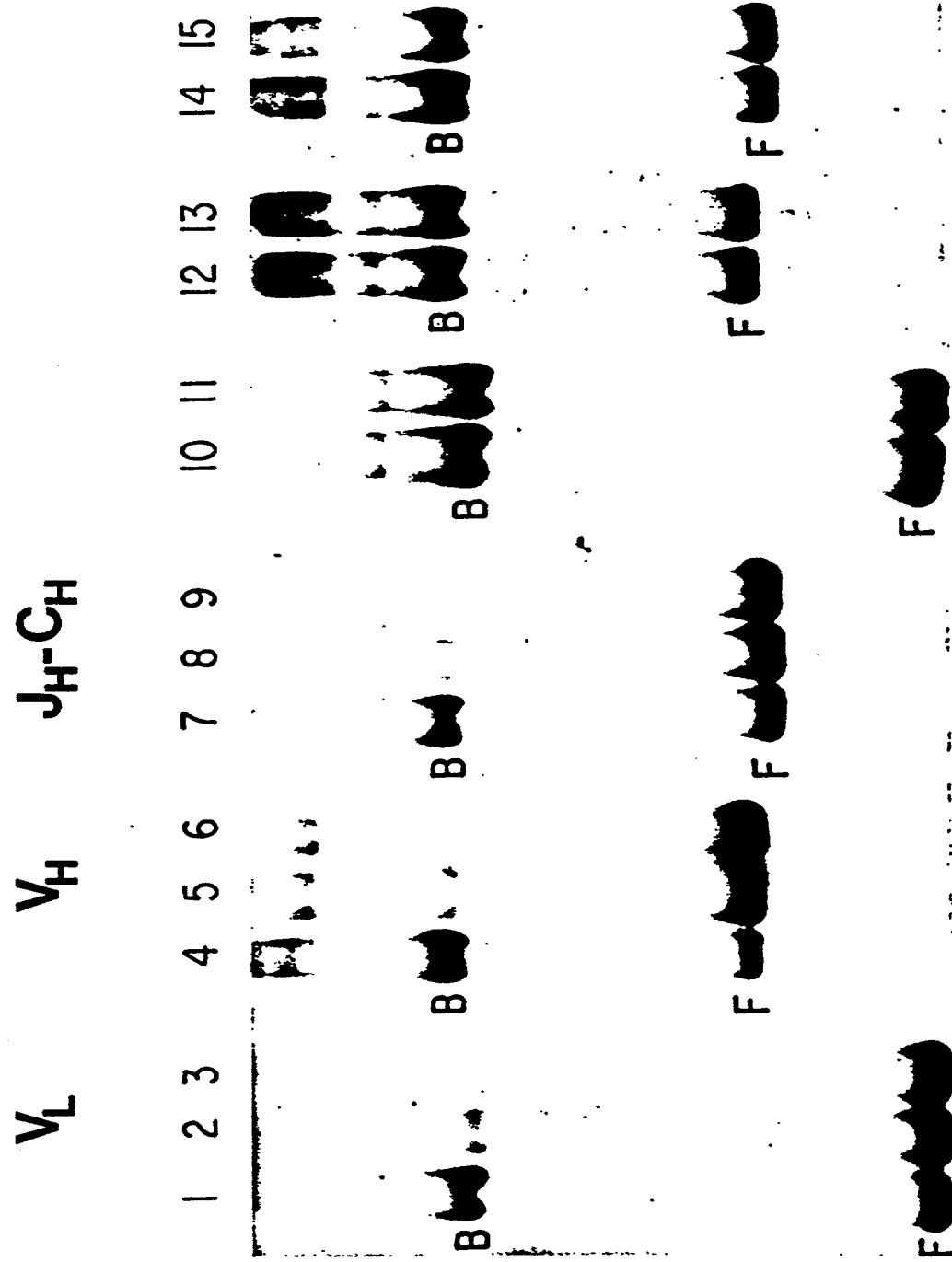


FIG.4A

V _L coding strand (-66)	*	TCTTAATA	ATTTGCAT	ACCCTCAC
V _H non-coding strand (-50)		CGCACATG	ATTTGCAT	ACTCATGA
J _H - C _μ coding strand (166)		CCTGGGTA	ATTTGCAT	TTCTAAAA

FIG. 4B



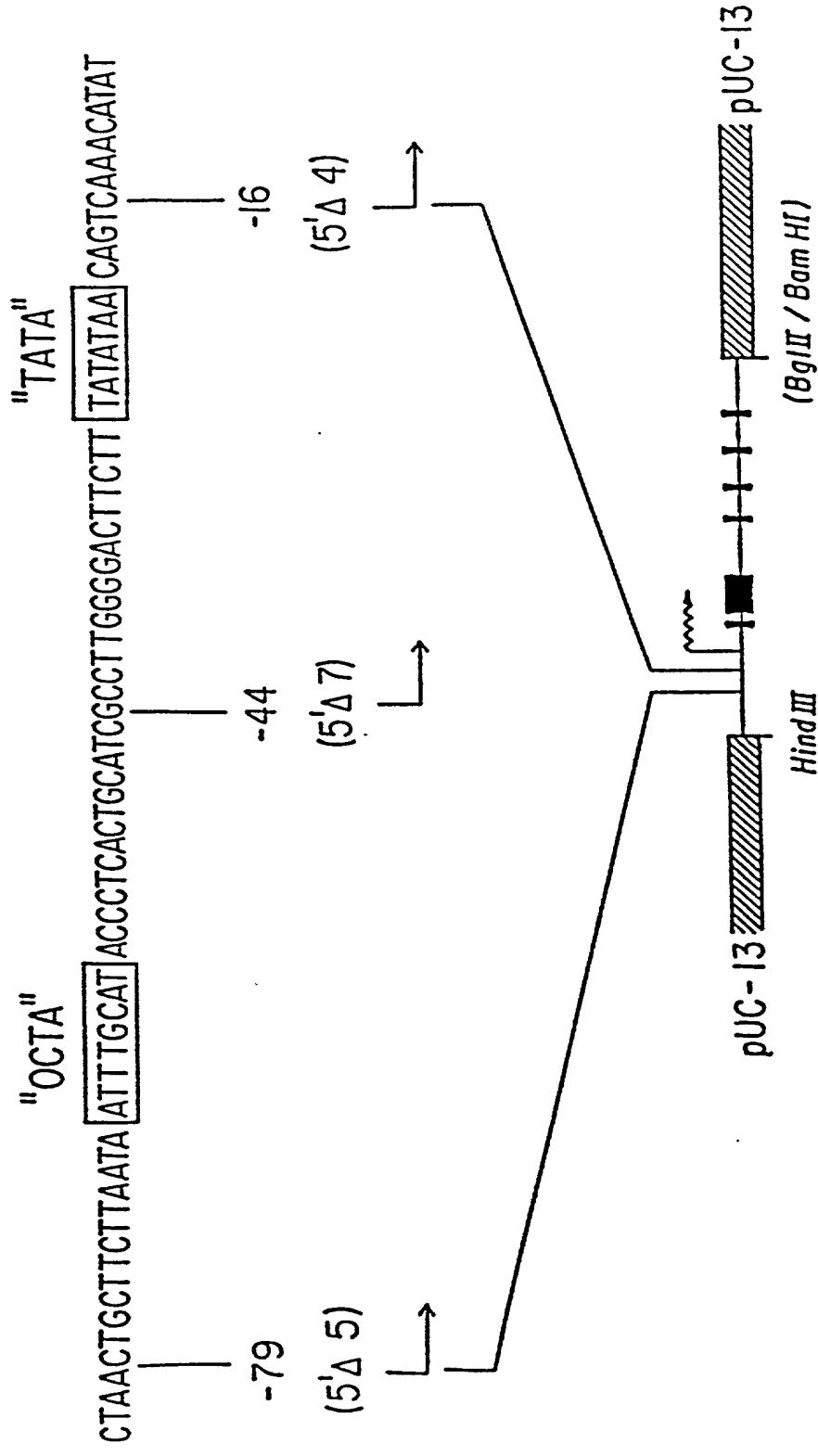


FIG. 5A

pK

FIG. 5B

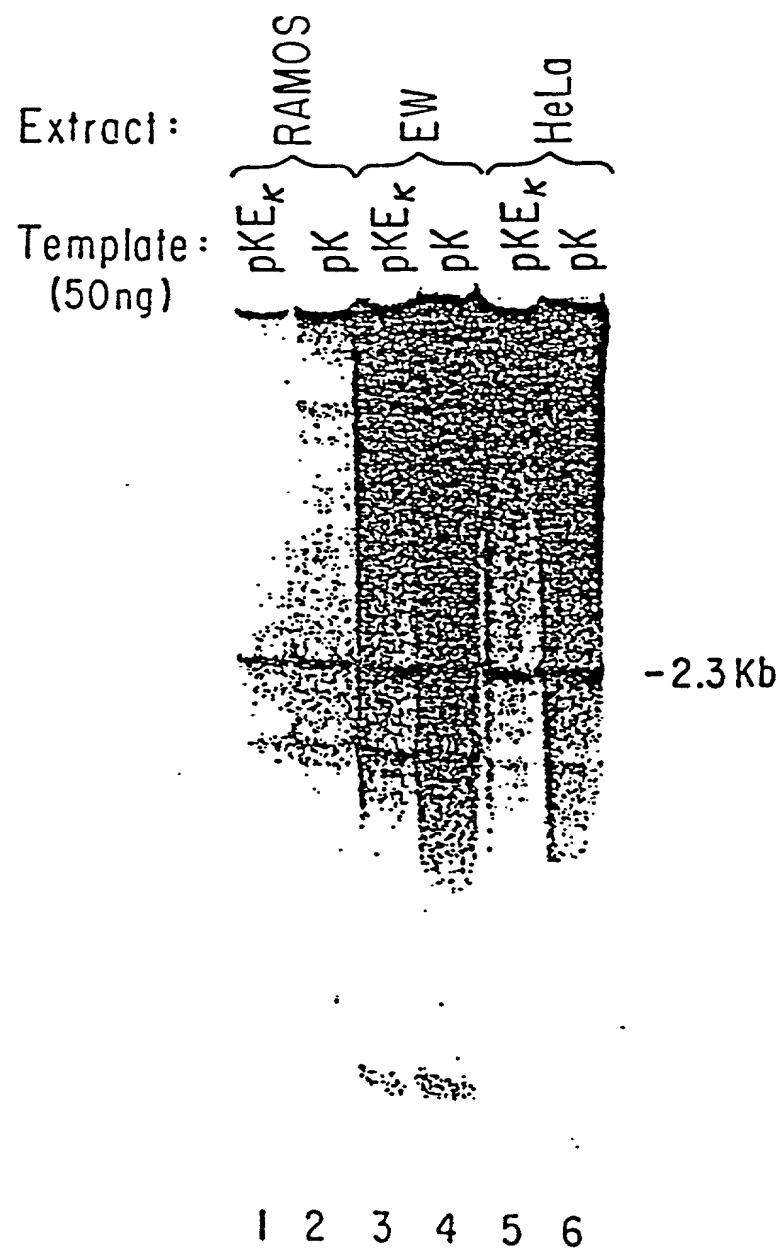


FIG.6

Extract:

Template:

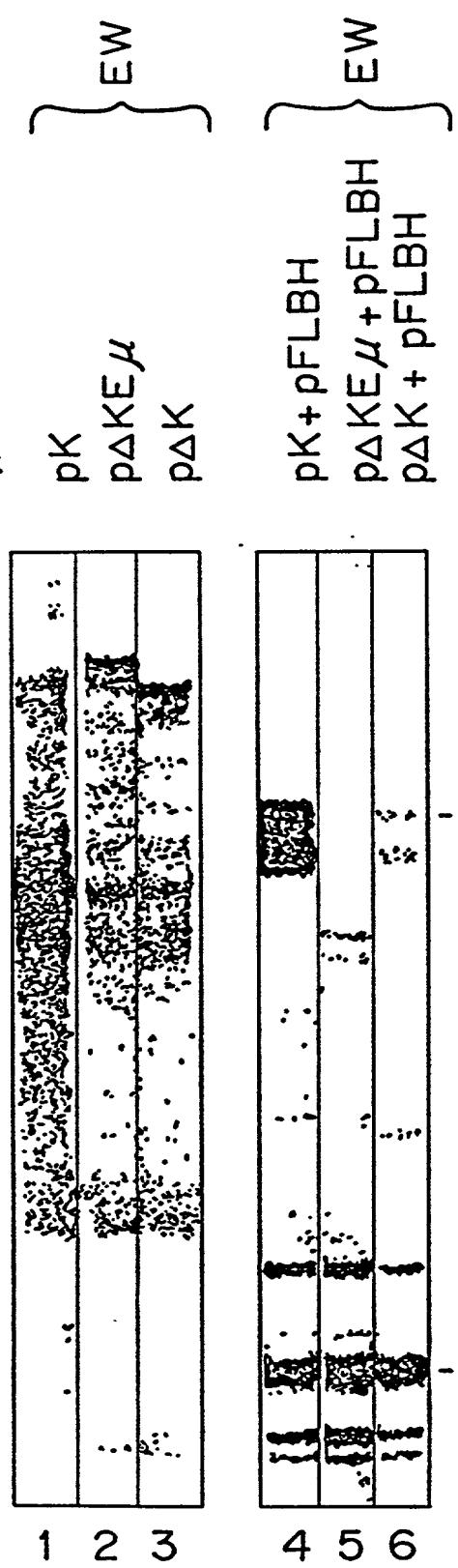
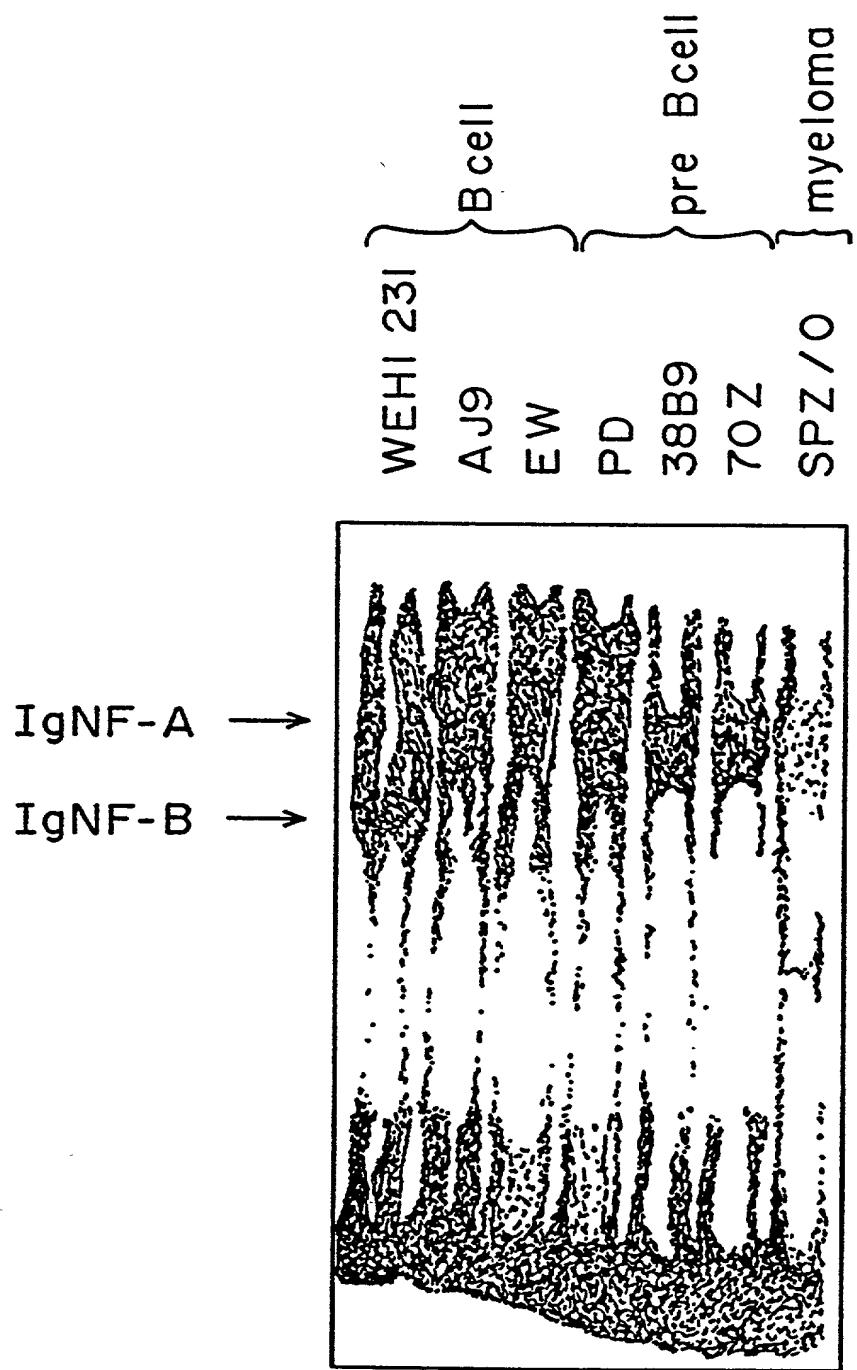
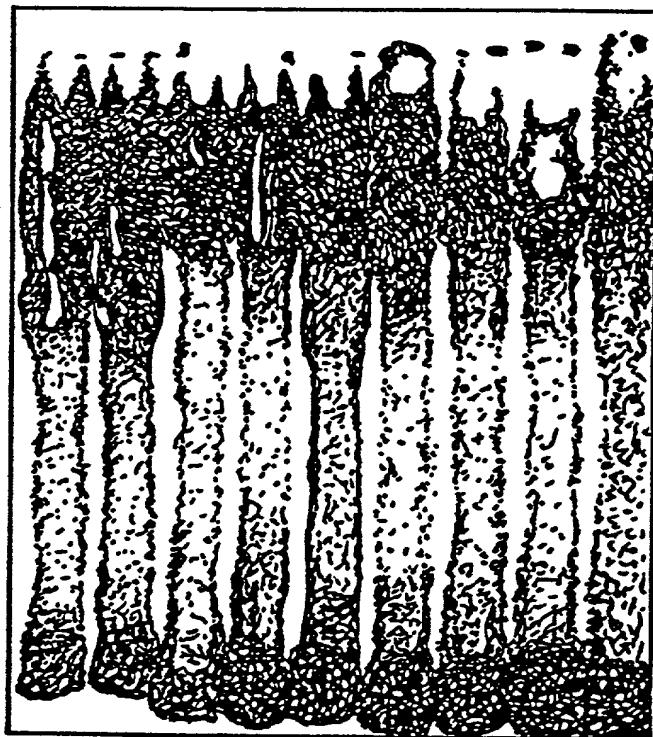


FIG. 7



IgNF-A →
IgNF-B →

WEHI 231 }
EL 4 } Tcell T
BW5147 } G.8
W7 }
RL^{O¹¹} }
HeLa }
 ψ 2 }
MEL }
COS



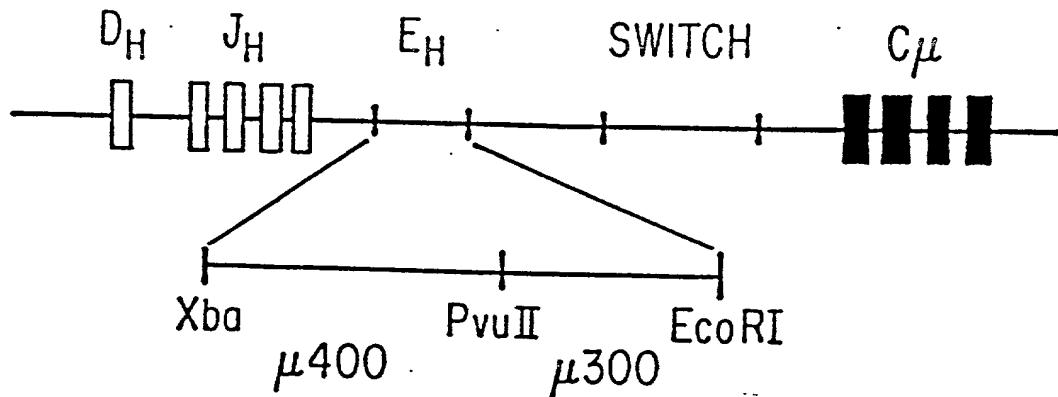


FIG.9A

Probe: μ 300
Extract: EW / N
Competitor:

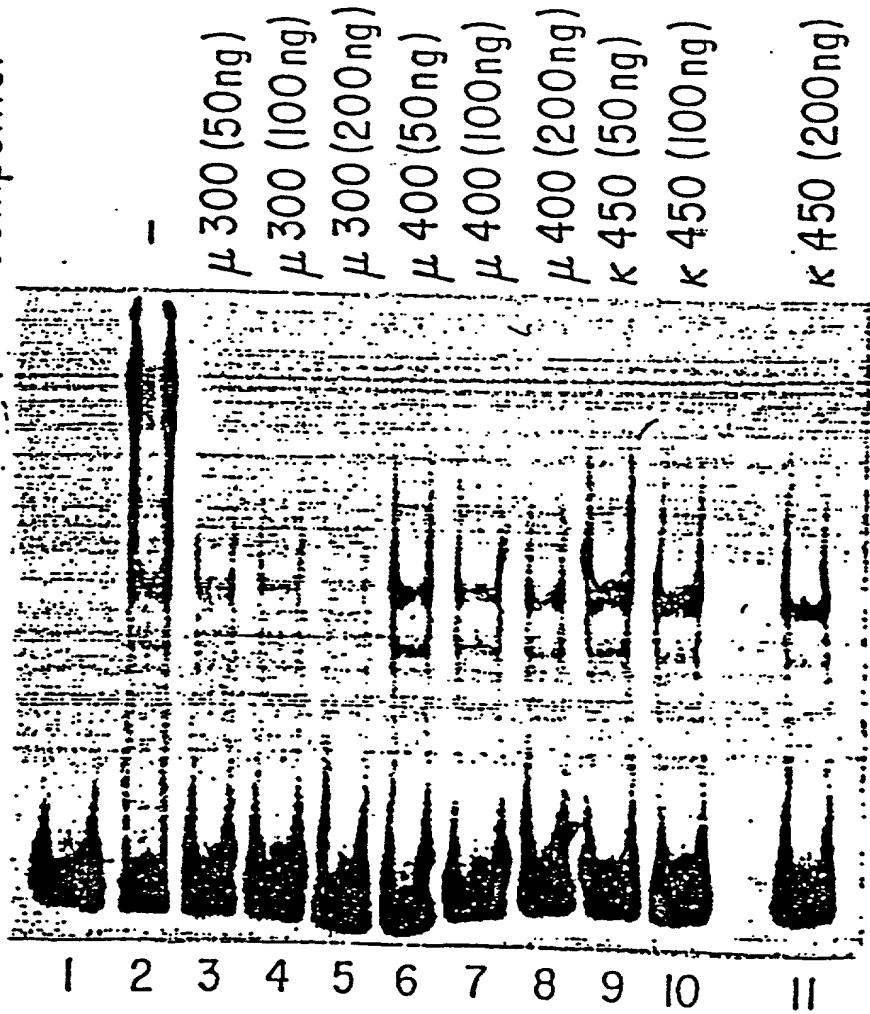


FIG.9B

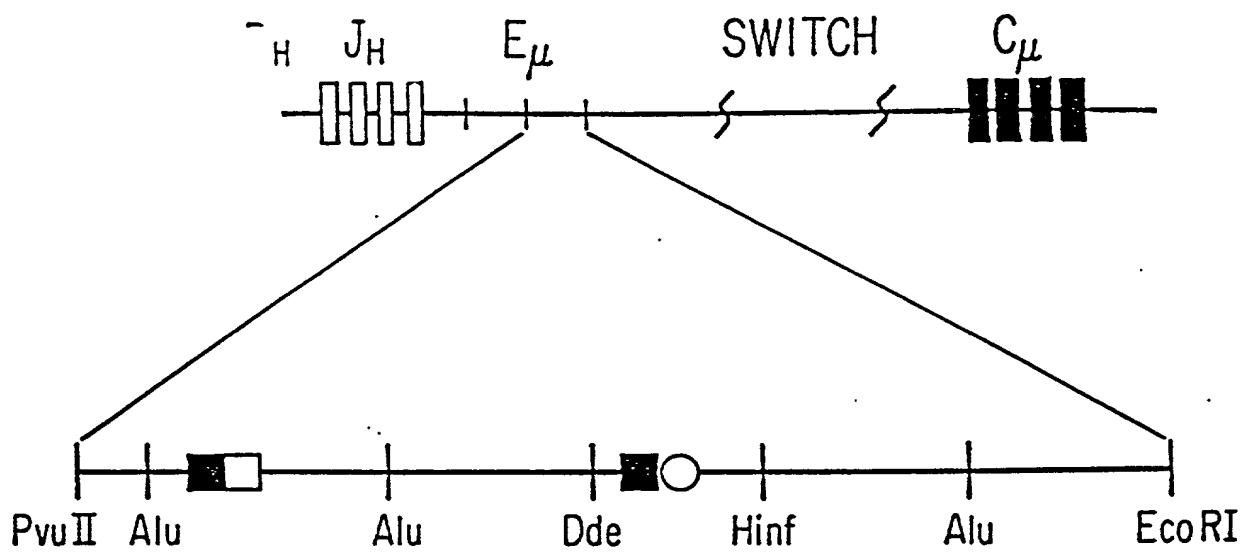


FIG.10A

E

□ : ?

O : Octamer (ATTTGCAT)

FIG. IOB

Probe: μ 50
- d.I.C.

$(\mu 60)_2$
dI.C.

μ 70
dI.C.

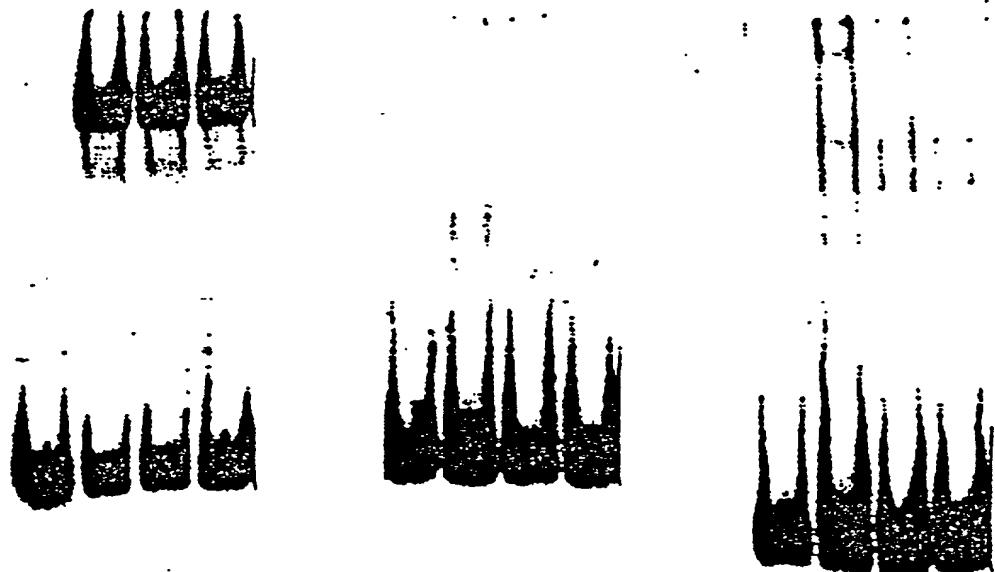


FIG. IOC

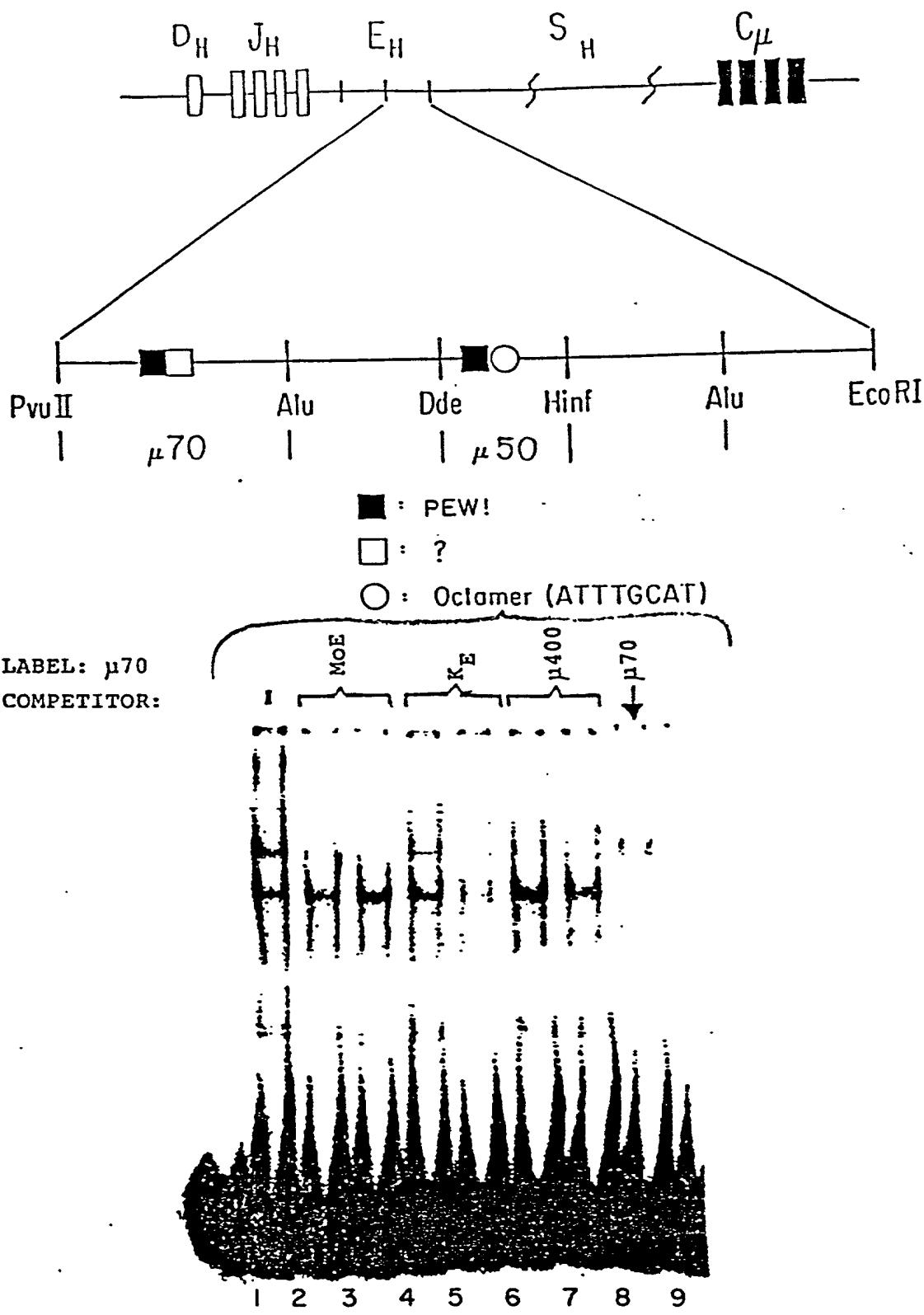


FIG. IOD

FIG.10E

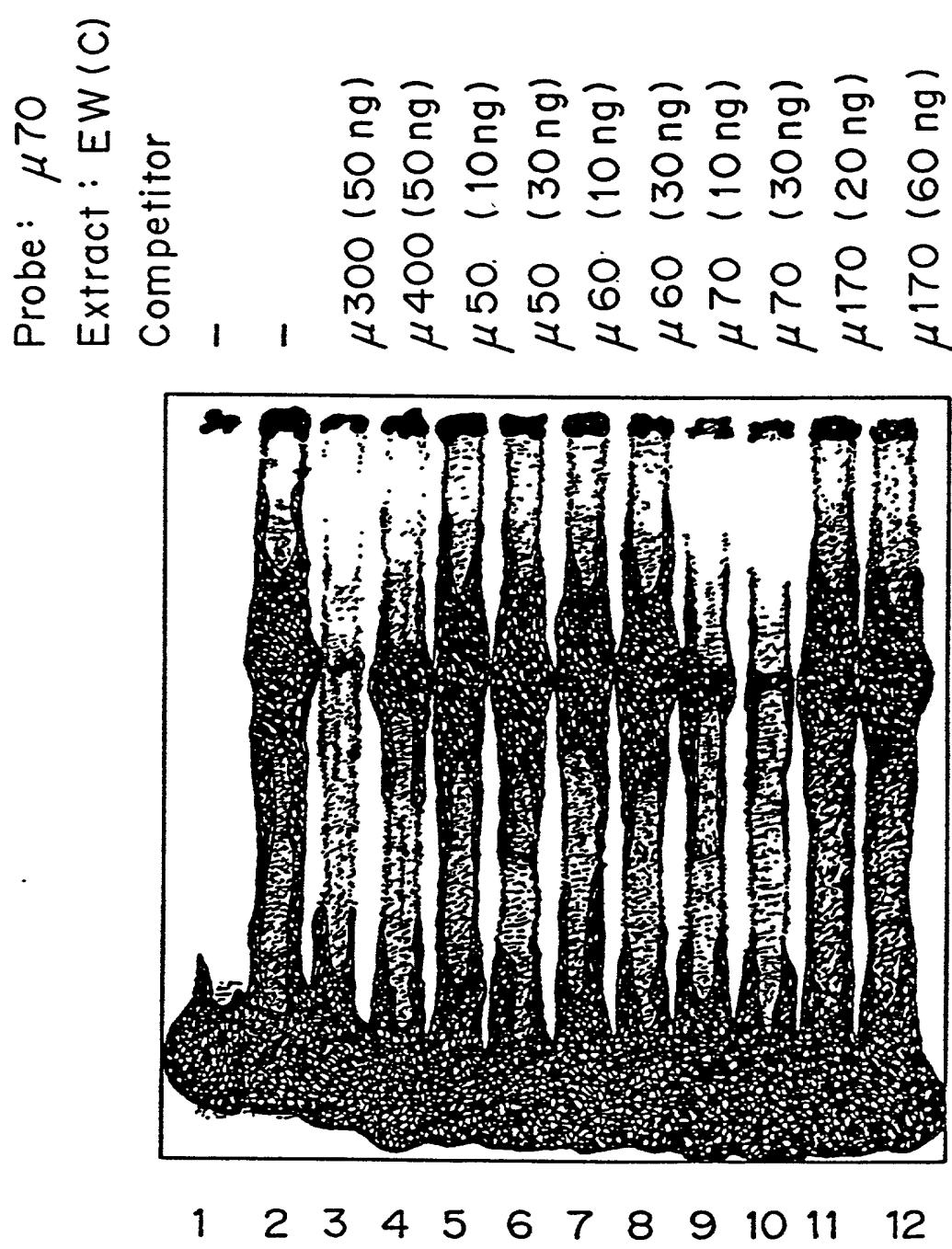


FIG.IIA

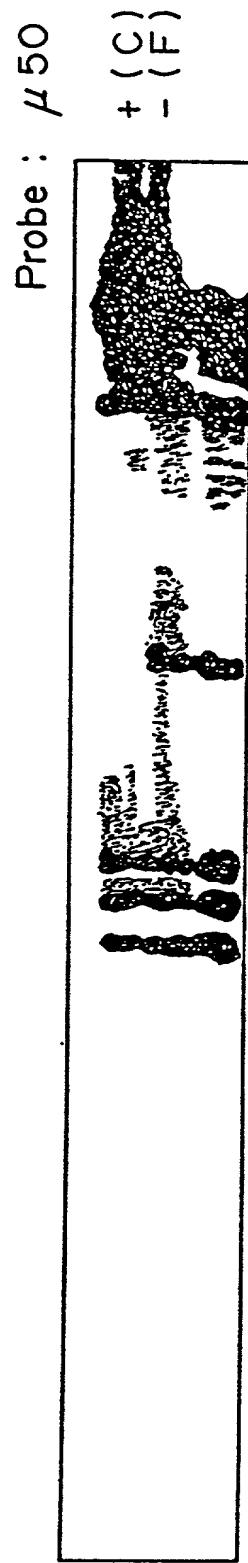
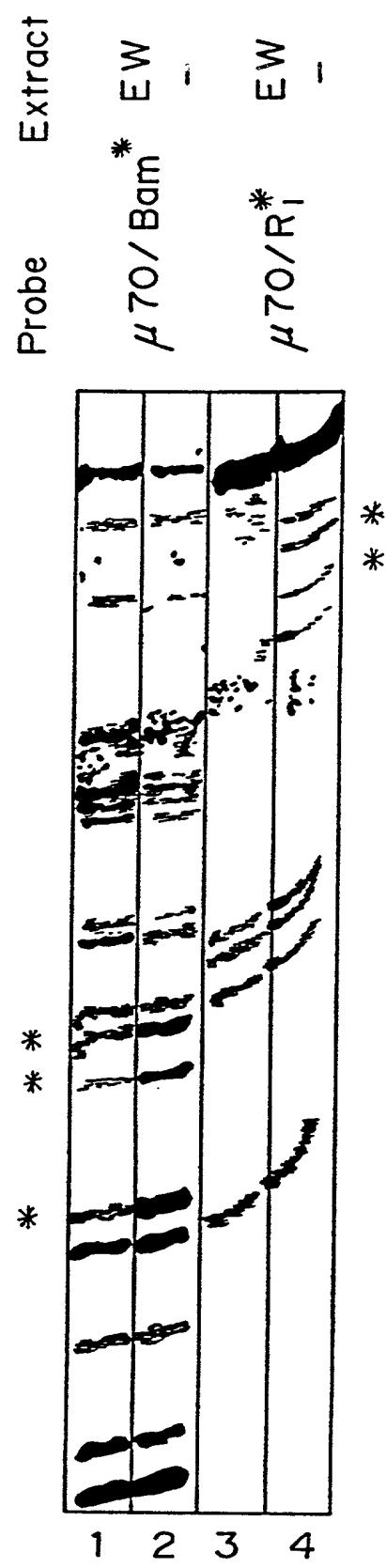


FIG.IIB



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AATTACCCAGTGGTGTGTTGCG
TTAATGGTCACCAACAAACG

170:

A G C A G @ T C A T @ T @ G C A A G G C T A
T C G T C C A @ T A C A C C @ T T C C G A T

FIG. II

FIG.I2A

1
2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19



FRAGMENT: μ 50
EXTRACT (9-11 μ gm)

HAF TL
PD
3889
70Z
EW
WEIII23I
AJ9
SP2-O
KR-12
8226
RL dII
W7
EL4
BW
COS
3T3
MEL
MeLa

1
2
3
4
5
6
7
8
9
10
11
12



FRAGMENT: μ 70
EXTRACT (9-11 μ g)

EW
C5
3889
70Z
WEIII
SP2-O
COS
3T3
MEL
PCC4
HeLa

FIG.I2B

FIG.13A

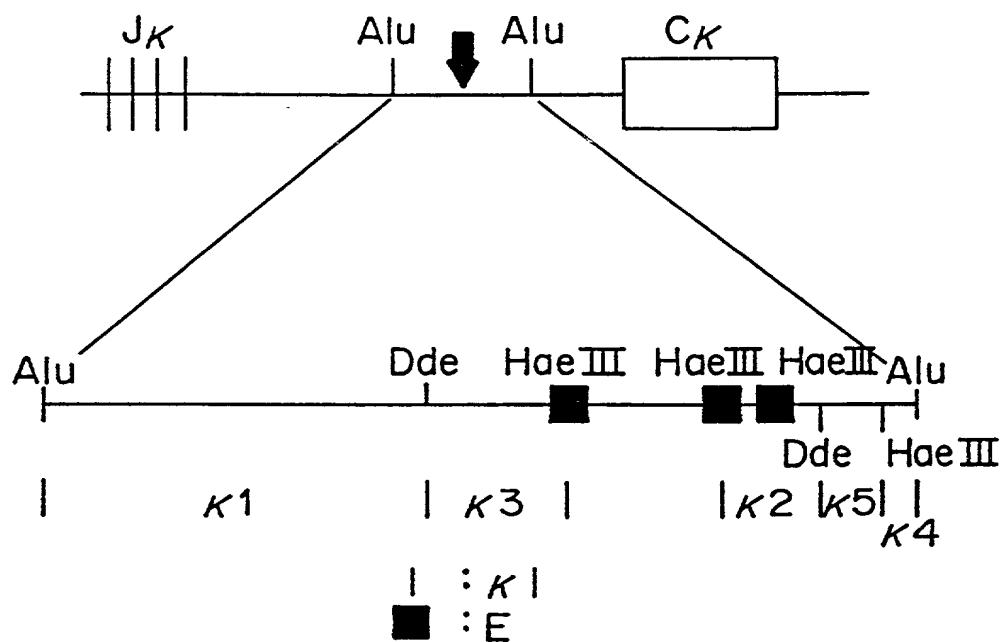


FIG.13B

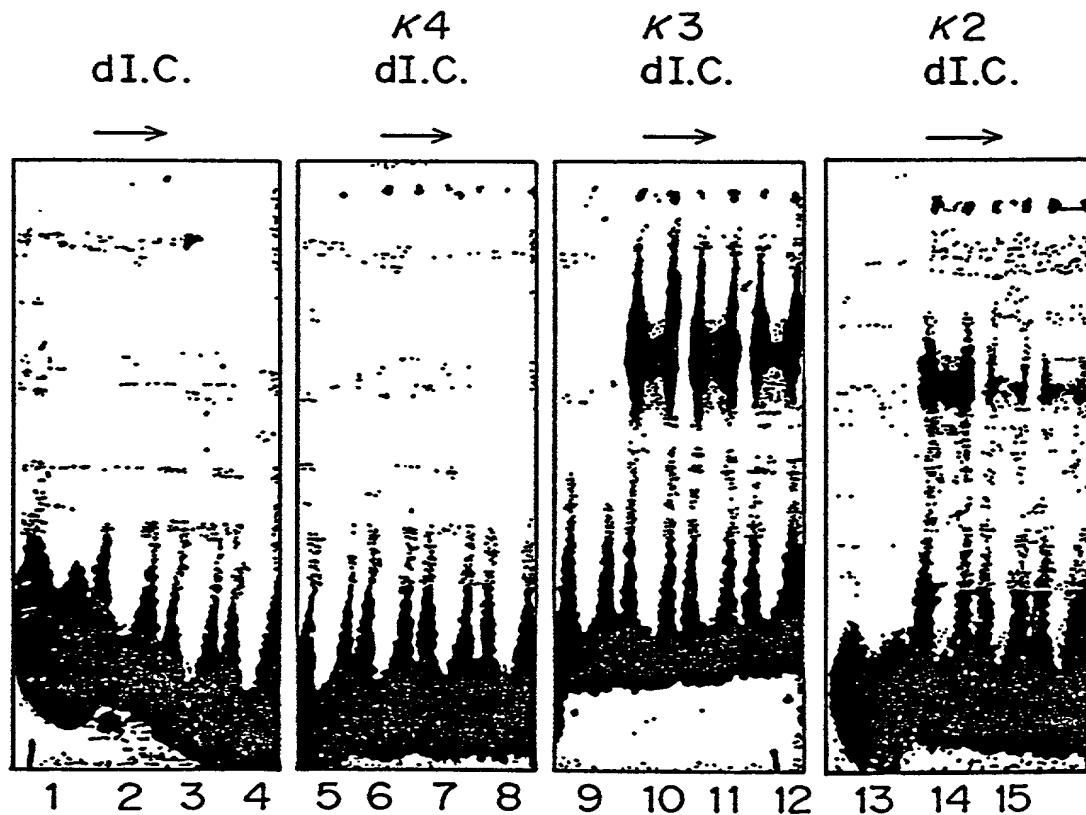
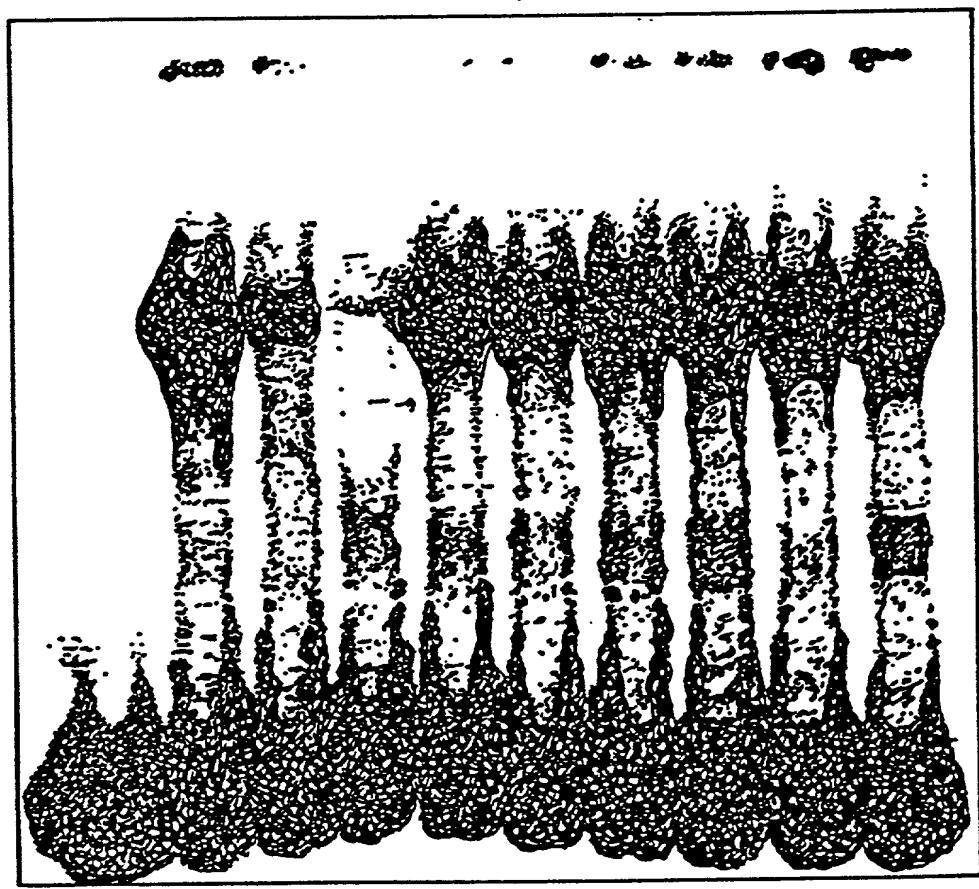


FIG. 13C

Extract EW/c 1 μ l
fragment Comp



1 2 3 4 5 6 7 8 9 10

FIG.13D

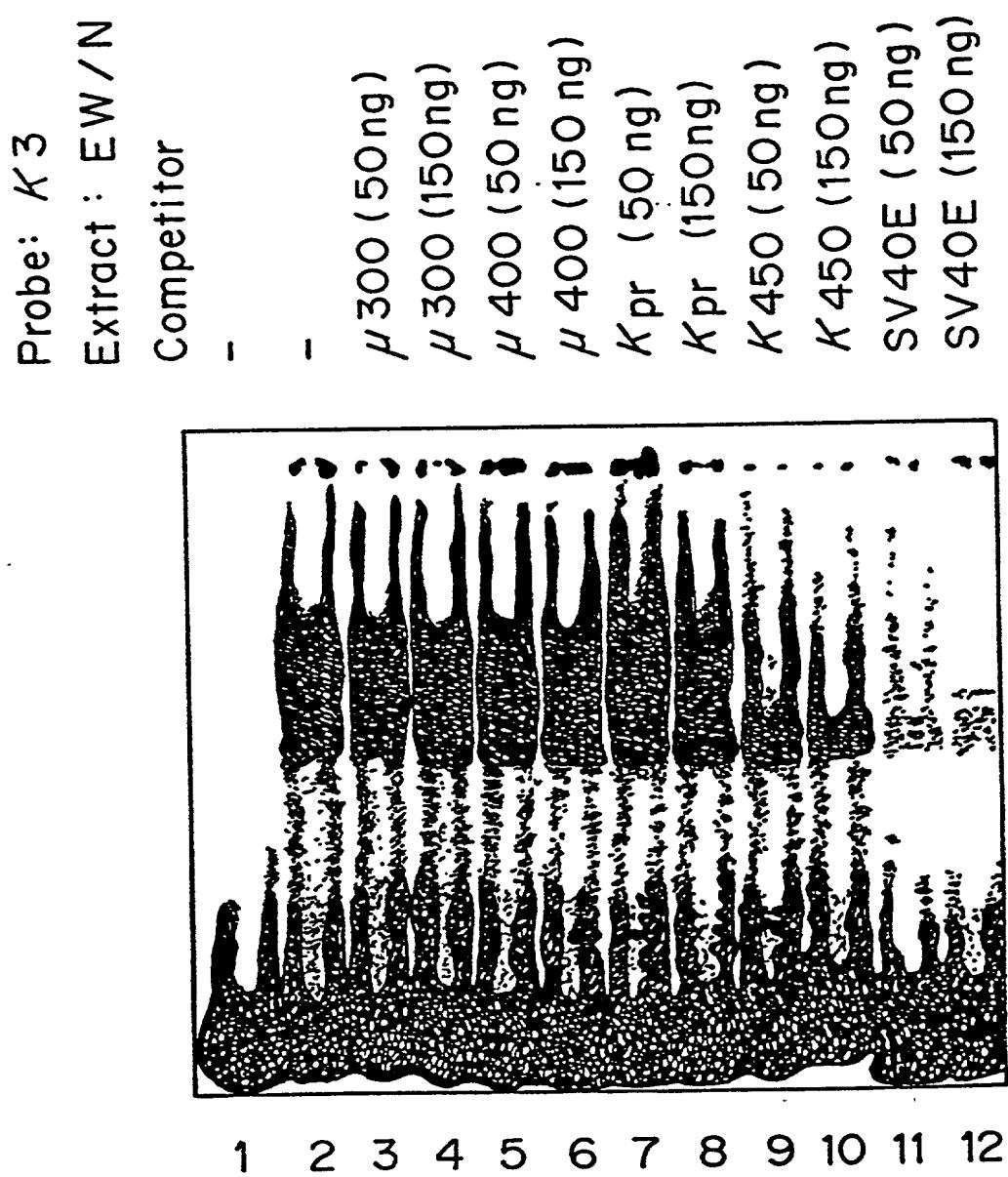


FIG.14

Probe : $K-3 / Dde^*$

Extract

MPC II

-

WEHI 23I

-



1 2 3 4

FIG.15A

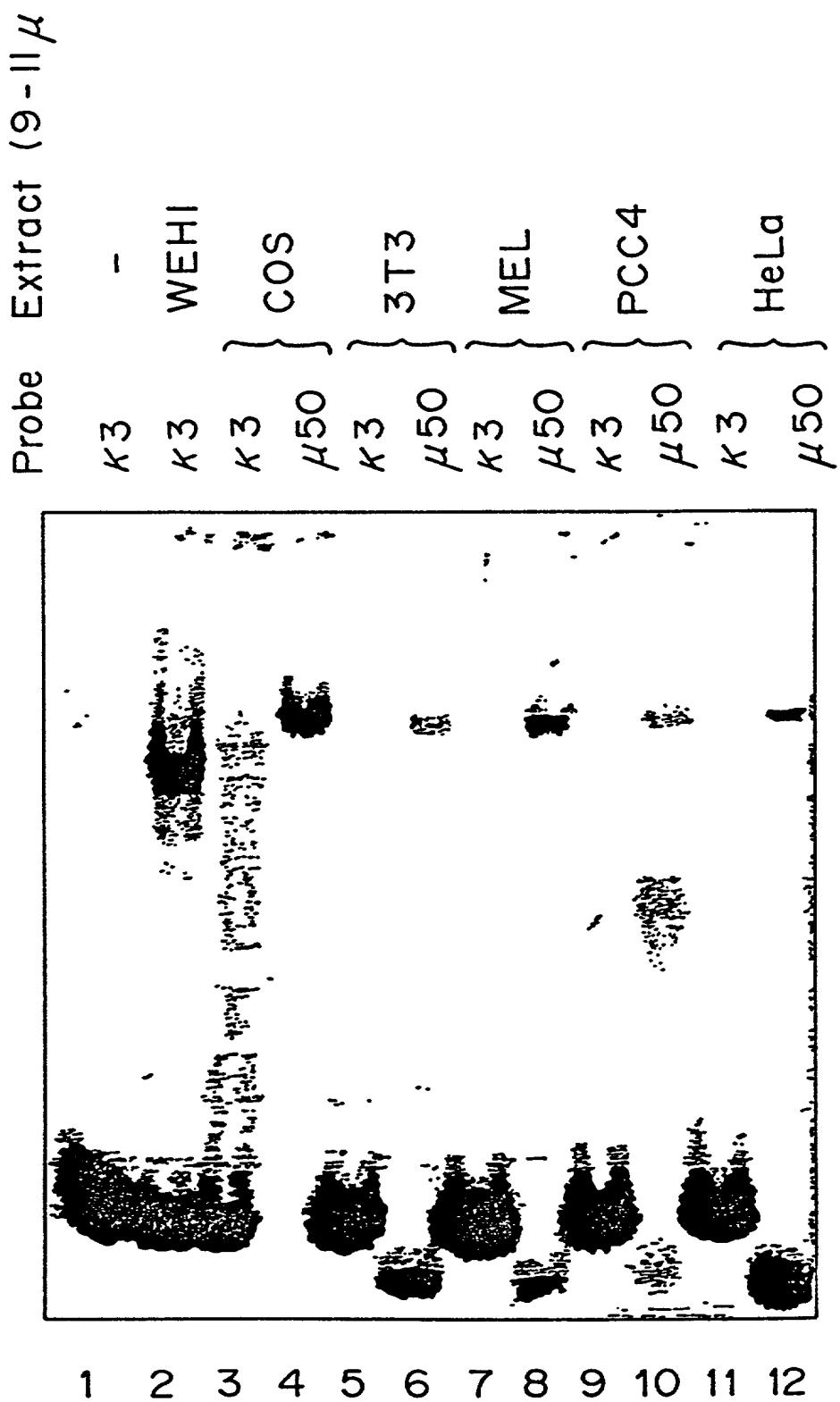


FIG. I-5B

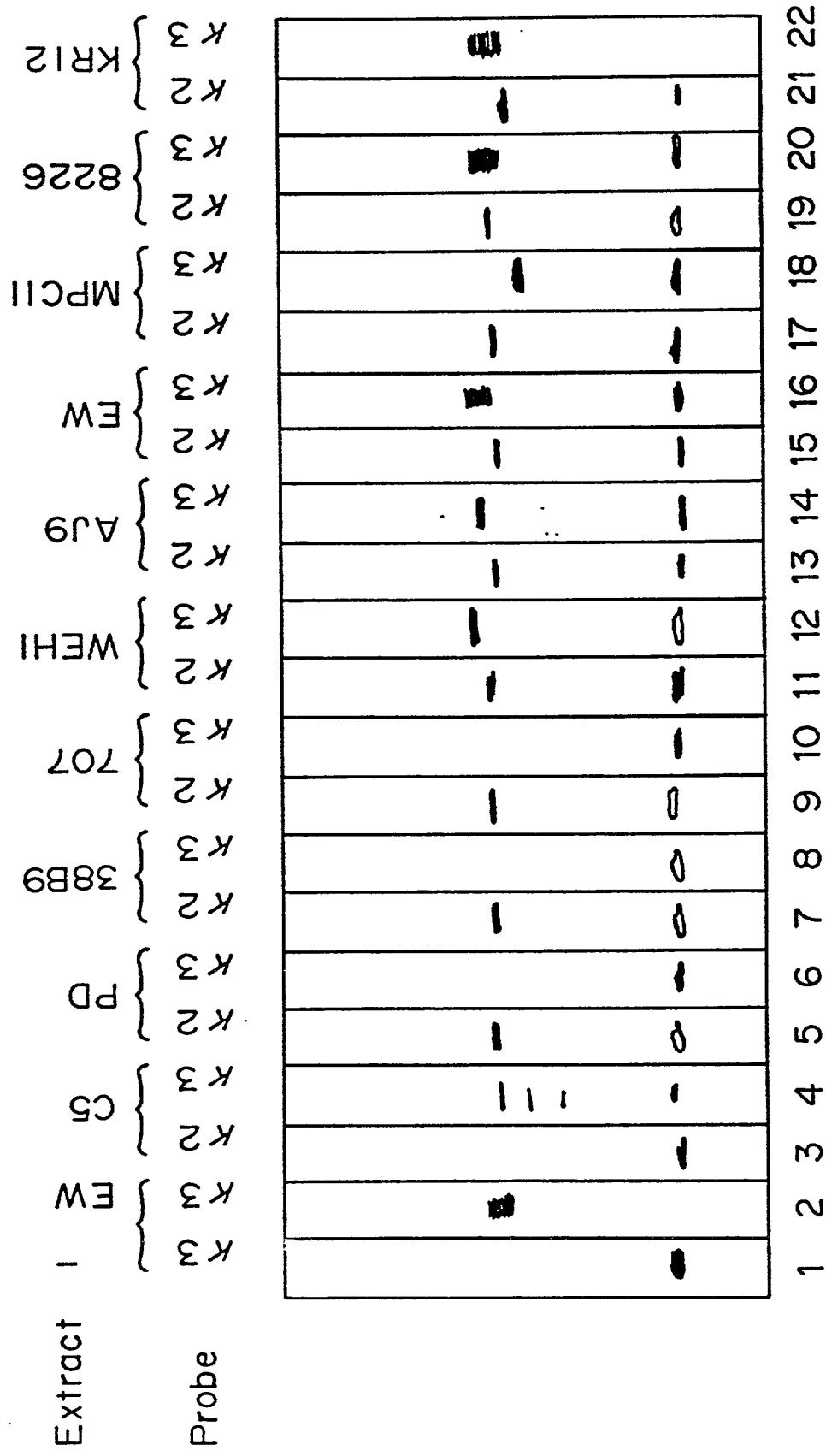


FIG.16

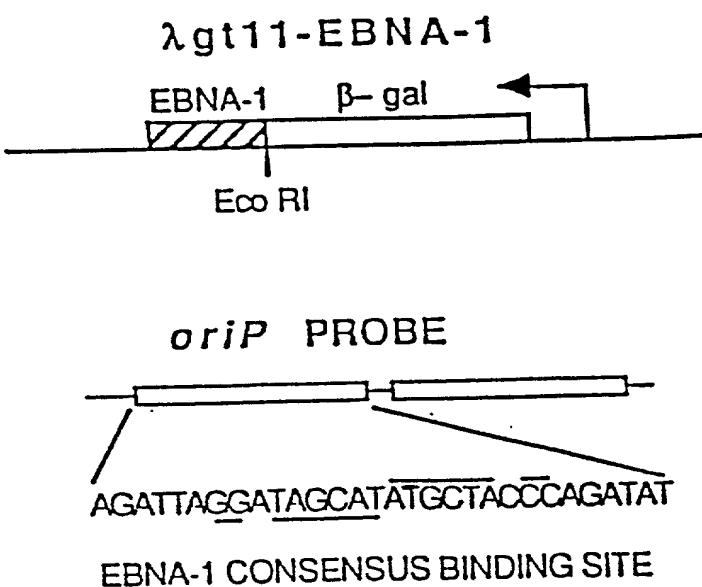


FIG.17

A.

<i>MHC</i>	T <u>GGGGATTCCCCA</u>
<i>mhc1</i>	TG <u>cGGATTCCCCaA</u>
<i>κEN</i>	a <u>GGGGActttCCg</u>
<i>κen</i>	aa <u>attActttCCg</u>
<i>SVEN</i>	T <u>GGGGActttCCA</u>
<i>HIV</i>	T <u>GGGGActttCCA</u>
	aa <u>GGGActttCCg</u>

1 CTGGGGCCCCAGAGAGGGTGGGAGATGACACAGTTCTCCCCAGCCCTGGCGGGCG
 1 -----+-----+-----+-----+-----+-----+
 61 GGCAGCATGGTCACTCCAGCATGGGGCTCCAGAAATAAGAATGTCTAACGCCCTGGAG
 61 -----+-----+-----+-----+-----+-----+
 M V H S S M G A P E I R M S K P L E
 121 GCCGAGAACAGCAAGGTCTGGACTCCCCATCAGAGCACACAGACACCGAAAGAAATGGACCA
 121 -----+-----+-----+-----+-----+-----+
 A E K Q G L D S P S E M T D T E R N G P
 181 GACACTAATCATCAGAACCCCCAAAATAAGACCTCCCCATTCTCCGTGTCCCCAACTGGC
 181 -----+-----+-----+-----+-----+-----+
 D T N H O N P Q N R T S P F S V S P T G
 241 CCCAGTACAAAGATCAAGGCTGAAGACCCCAGTGGCGATTAGCCCCAGCAGCACCCCTG
 241 -----+-----+-----+-----+-----+-----+
 P S T K I K A E D P S G D S A P A A P L
 301 CCCCTCAGCCGGCCAGCCTCATCTGCCCAAGGCCAACTCATGTTGACGGGCAGCCAG
 301 -----+-----+-----+-----+-----+-----+
 P P Q P A Q P N L P Q A Q L M L T G S Q
 361 CTAGCTGGGACATACAGCAGCTCCTCCAGCTCCAGCAGCTGGTGCTTGTGCCAGGCCAC
 361 -----+-----+-----+-----+-----+-----+
 L A G D I Q Q L L Q L Q Q L V L V P G H
 421 CACCTCCAGCCACCTGCTCAGTCCTGCTACCGCAGGCCAGCAGGCCAGGCCAGCTG
 421 -----+-----+-----+-----+-----+-----+
 H L Q P P A Q F L L P Q A Q Q S Q P G L
 481 CTACCGACACCAAATCTATTCCAGCTACCTCAGCAAACCCAGGGAGCTTCTGACCTCC
 481 -----+-----+-----+-----+-----+-----+
 L P T P H L F Q L P Q Q T Q G A L L T S
 541 CAGCCCCGGGCCGGCTTCCCACACAGGCCGTGACCCGCCCTACGCTGCCCGACCCGCAC
 541 -----+-----+-----+-----+-----+-----+
 Q P R A G L P T Q A V T R P T L P D P H
 601 CTCTCGCACCGCAGCCCCCAAATGCTGGAGCCACCATCCCACCCGAGGAGCCAGT
 601 -----+-----+-----+-----+-----+-----+
 L S H P Q P P K C L E P P S H P E E P S
 661 GATCTGGAGGGAGCTGGAGCAATTGGCCCGACCTTCAAGCAACGCCGCATCAAGCTGGC
 661 -----+-----+-----+-----+-----+-----+
 D L E E L E Q F A R T F K Q R R I K L G
 721 TTCACGCAGGGTGATGTGGGCTGGCCATGGCAAGCTCTACGCCAACGACTTCAGCCAG
 721 -----+-----+-----+-----+-----+-----+
 F T Q G D V G L A M G K L Y G N D F S Q
 C G P G H G Q A L R Q R L Q P D

FIG. 18A

1261 GTTACTACCTTATCCTCAGCTGTGGGGACGCTCCACCCCAGCCGGACAGCTGGAGGGGGT
 V T T [L] S S A V G T [L] H P S R T A G G G
 Y Y L I L S C G D A P P Q P D S N M G W

1321 GGGGGCGGGGGCGGGGCTGCGCCCCCTCAATTCCATCCCCCTGTCACTCCCCCACCC
 G G G G G A A P P L N S I P S V T P P P
 G M G R G C A P P Q F H P L C H S P T P

1381 CCGGCCACCAACAAACAGCACAAACCCAGCCCTCAAGGCAGCCACTCGGTATCGGCTTG
 P A T T N S T N P S P Q G S H S A I G L
 G H N Q Q H K P Q P S R Q P L G Y M L V
 TCAGGCCTGAACCCCAGCACGGGTAAGTGGGTGCACGTGGGAAGCTGTGGGGAGAAGCA
 1441 S G L H P S T G +
 A P E P Q N G V S G C T W E A V G R S R

1501 GCGTCGCTGCTCTTCTAGGGTGGGAGCGGCACCCAGTTATGTTGGCAGGTCCCTGCC
 V A A A S R V G S G T P V M L A G P C P

1561 CCTGCTAATGCCTCTGCTTGCCTCTGCAGAACATGGTGGGTTGAGCTCCGGCT
 C +

1621 GAGTCCAGCCCTCATGAGCAACAACCCCTTGGCCACTATCCAAGGTGCGTGCTGCCATC
 1681 GTCACACCCATCGTCACCAGCCCCGGAATTGAG

FIG.18A (CONT.)

EQUUS HYBRID C14

```

ACGACCATTCCCGCTTCGAGGCCCTAACCTGAGCTCAAGAACATGTGCAAACCAAG
781 T T I S R F E A L N L S F K N M C K L K
D H F P L R G P Q P E L Q E H V Q T Q A

CCCCTCCTGGAGAAGTGGCTAACGATGCAGAGACTATGTCTGTGGACTCAAGCCTGCC
841 P L L E K W L N D A E T M S V D S S L P
P P G E V A Q R C R D Y V C G L K P A Q

AGCCCCAACCAGCTGAGCAGCCCCAGCCTGGTTTCGAGCCTGCCGGCCGGAGACGCAAG
901 S P N O L S S P S L G F E P A G R R R K
P Q P A E Q P Q P G F R A C M P E T Q E

AAGAGGACCAGCATCGAGACAAACGTCCGCTCGCCTAGAGAAGAGTTTCTAGCGAAC
961 K R T S I E T N V R F A L E K S F L A N
E D Q M R D K R P L R L R E E F S S E P

CAGAAGCCTACCTCAGAGGAGATCCTGCTGATGCCGAGCAGCTGCACATGGAGAAGGAA
1021 Q K P T S E E I L L I A E Q L H M E K E
E A Y L R G D P A D R R A A A H G E G S

GTGATCCCGGTCTGGTTCTGCAACCGGCCCCAGAAGGACAAACGCATCAACCCCTGCAGT
1081 V I R V W F C N R R Q K E K R I H P C S
D P R L V L Q P A P E G E T H Q P L Q C

GCGGCCCATGCTGCCAGCCAGGGAAAGCCGCCAGCTACAGCCCCATATGGTCACA
1141 A A P M L P S P G K P A S Y S P H H V T .
G P H A A Q P R E A G Q L Q P P Y G H T

CCCCAAGGCAGGCCGGGACCTTACCGTTCTCCAAGCTTCCAGCAGCTTGAGCACACA
1201 P Q G G A G T L P [L] S Q A S S S [L] S T T
P A G R G D L T V V P S F Q Q S E H N S

```

FIG.18A (CONT.)

100% G+C at 40°C

1411 CCTCAAGGCAGCCACTCGGCTATCGGCTTGTCAAGCCTGAACCCCAGCACGGGCCCTGGC
P Q G S H S A I G L S G L N P S T G P G
S A Q P L G Y R L V M P E P Q M G P N P

1471 CTCTGGTGGAACCTGCCCTTACCAAGCCTGATGGCAGCGGGAACTGGTGCTGGGGGC
L W W N P A P Y Q P .
L V E P C P L P A L M A A G I W C W G Q

1531 AGCCGGTGCAGCCCCGGGAGCCCTGGCCTGGTACCTCGCCGCTCTTCTGAATCATGC
P V Q P R G A L A W .

1591 TGGGCTGCCCTGCTCAGCACCCCGCCTGGTGTGGCCTGGTCTCAGCAGCGGCTGCAGGG

1651 TGTGGCAGCCTCCATCTCCAGCAAGTCTCCTGGCCTCTCCTCCTCATCCTCTTCATCCTC

1711 ATCCTCCTCCCTCCACTTGCAGCGAGACGGCAGCACAGACCCTGGAGGTCCAGGGGGG

1771 CCCGAGGCAGGGTCCAAACCTGAGTGAGGGCCAGCCATGCCTCCCTCCCATTCTCTGG

1831 TCCCTGCCCGGAATTC

FIG.18B

N	a	a	a	E/D		DNA	LLLL	C Oct-2
---	---	---	---	-----	--	-----	------	---------

N	LORF (277 AA)	C
---	---------------	---

FIG.18C

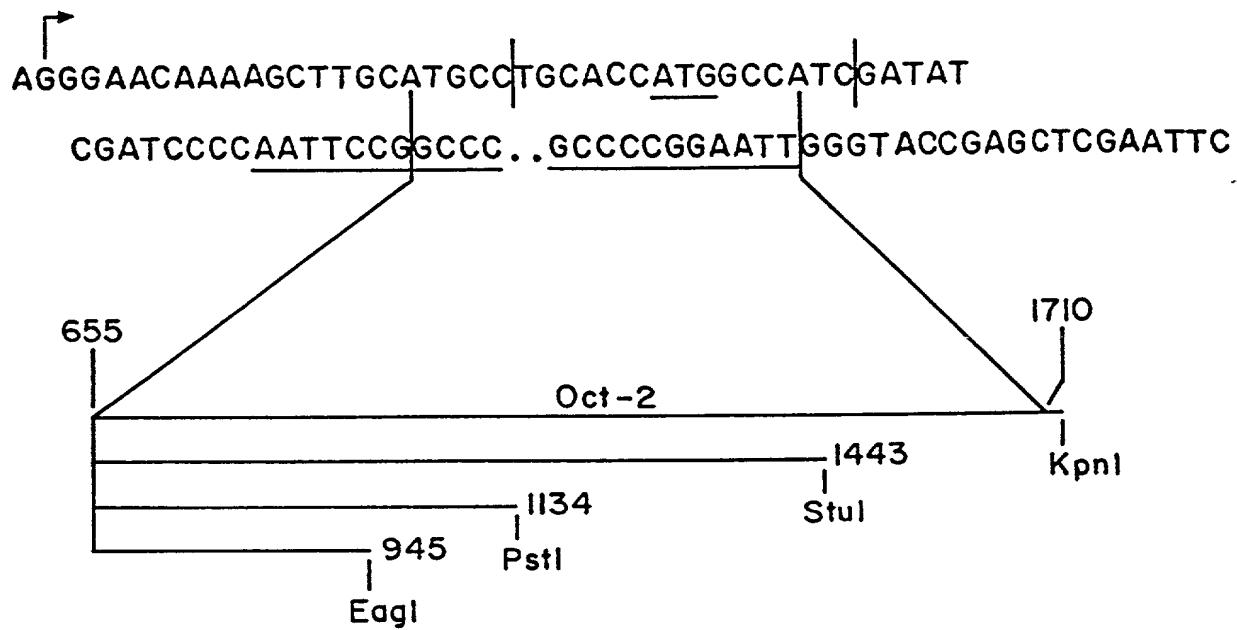


FIG.19

FIG.20

helix turn helix →

← Oct-2 PRKKRTSIETNVRF~~A~~E~~K~~SEFLANQKPTSEEILLIAEQLHMEKEVIRVWECMNRQKEKRINPC

* *

a1 SPKGKSSISPPQARAFLEQVERRKQSLSKEKEEVAKKKCGITPLQVRVHEINKMRSK

* *

α2 KPYRGHRFTKENVRILESWEAKNPYLDIKGLENLMKNTSISRIQIKNEWVSNRRBRKEKTIT

*

pho2 QRPKBRTRAKGEALDVLRKFEINPTPSLVERKKISDLIGMP EKNVRIWEONRRAKLRKKQ

* *

mec-3 RRGPFRTTIKQNQLDVLNEMFSNTPKPSKHARA~~K~~LAETGLSMRVIQVWEONRRESKERRLK

* *

cut SKKQQRVLFSEEQKEA~~R~~LAFALDPYPNVGTIEFLANELGIATRTITNWEHNHMRRLKQQV

* *

en EKRPTAFSSEQ~~L~~ARLKRE~~N~~ENRXYLTERRRQQLSELGINEAQIKIKEONKRAKIKKST

* *

Antp RKRCGROTYYTRYQTLEKEFHENRYLTRRRIEIAHALCLTERQIKIKEONRRMWKKKEN

* *

(conserved
residues in
homeo-box
family)

R	Q	I	Y	L	W	N	R
---	---	---	---	---	---	---	---

FIGURE 21A

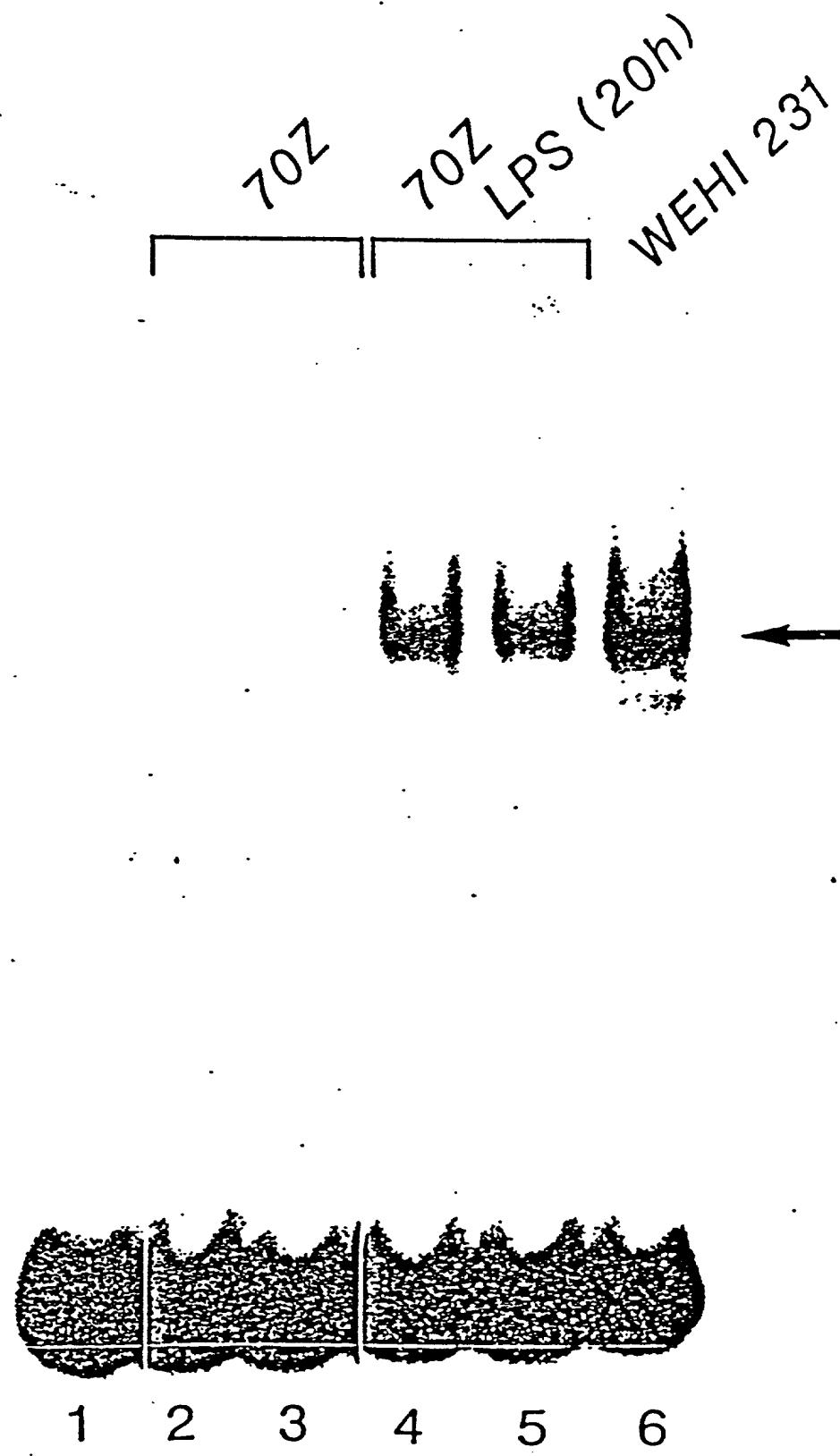
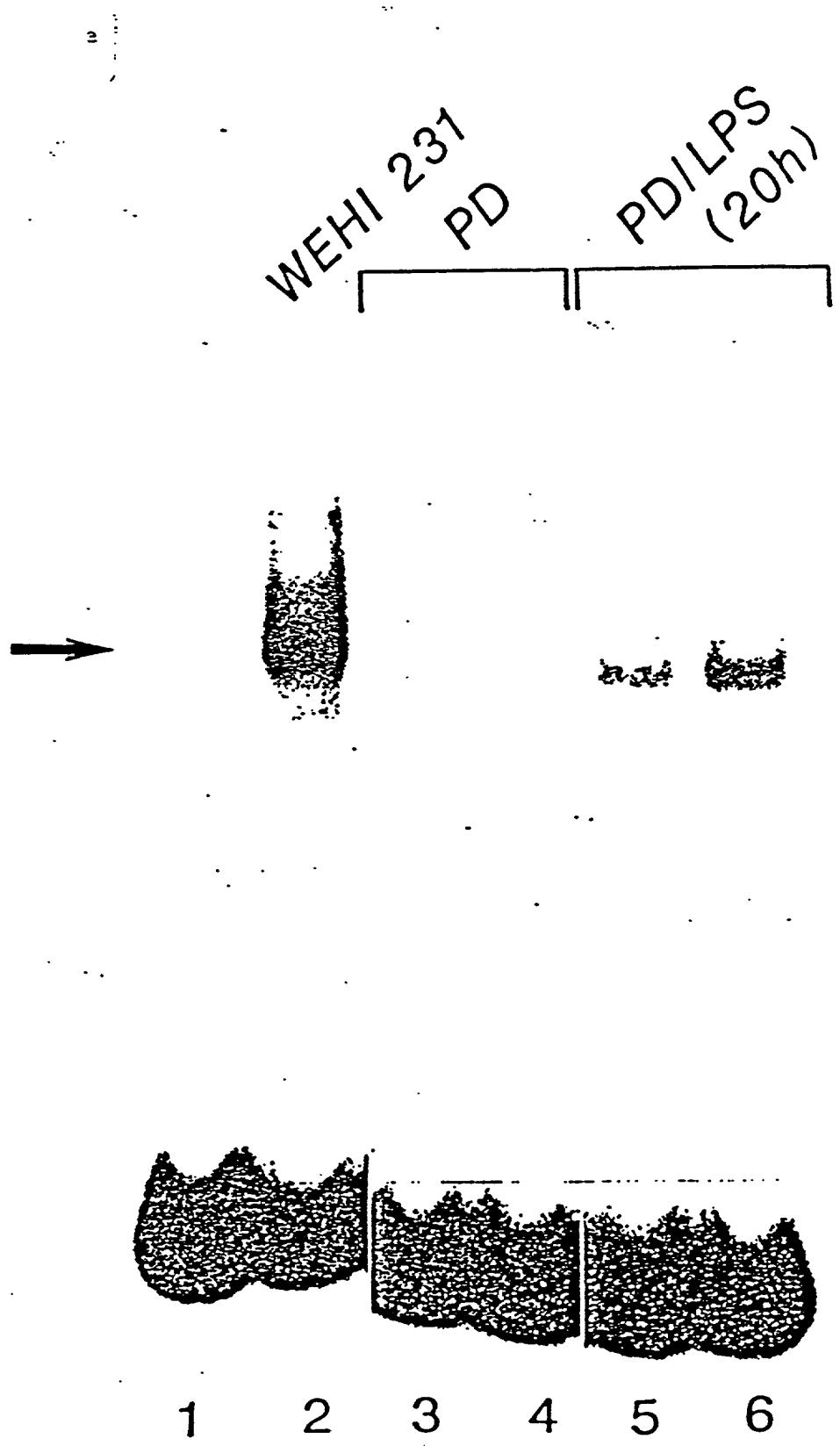


FIGURE 21B



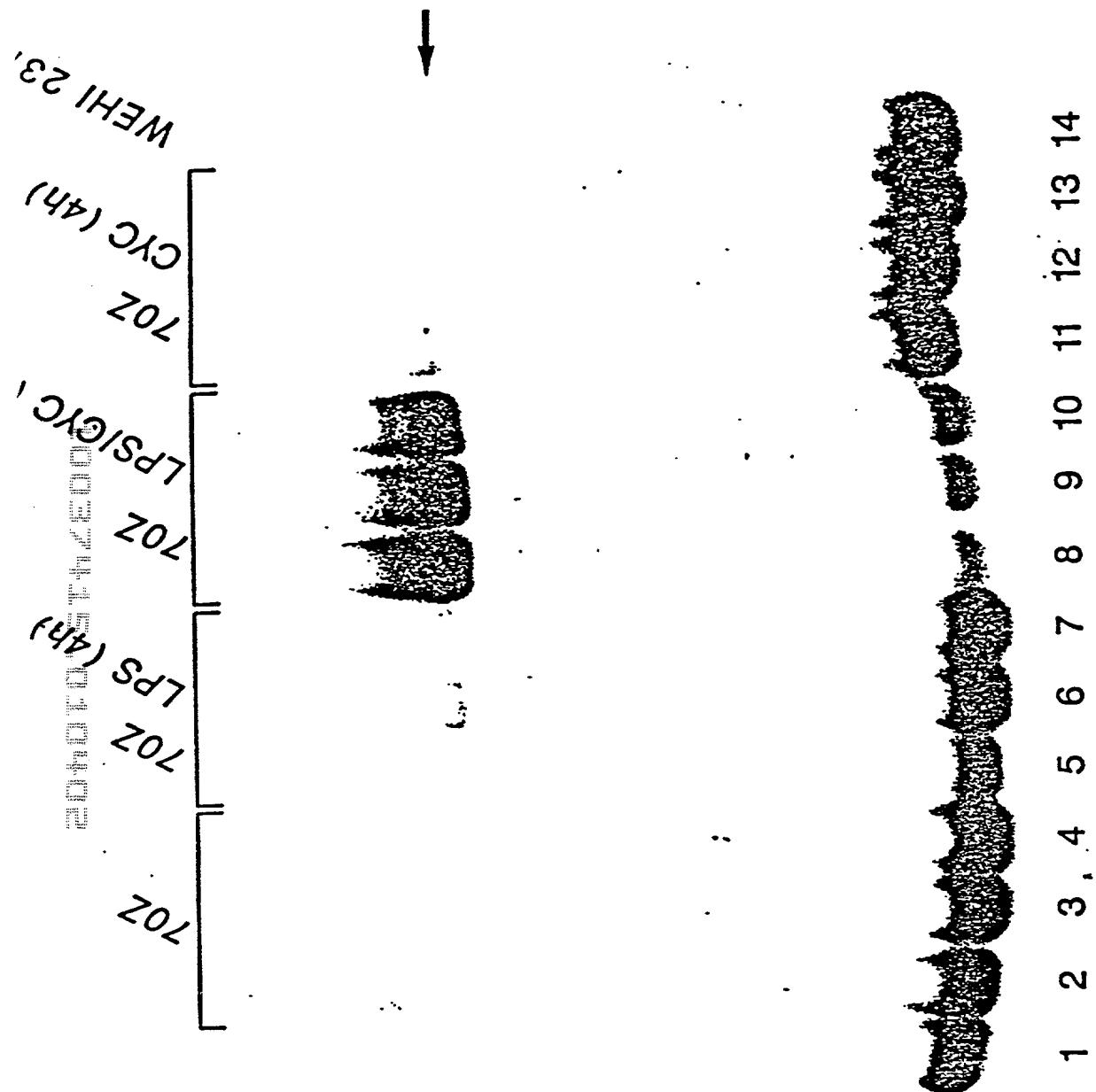


FIGURE 22A

FIGURE 22B

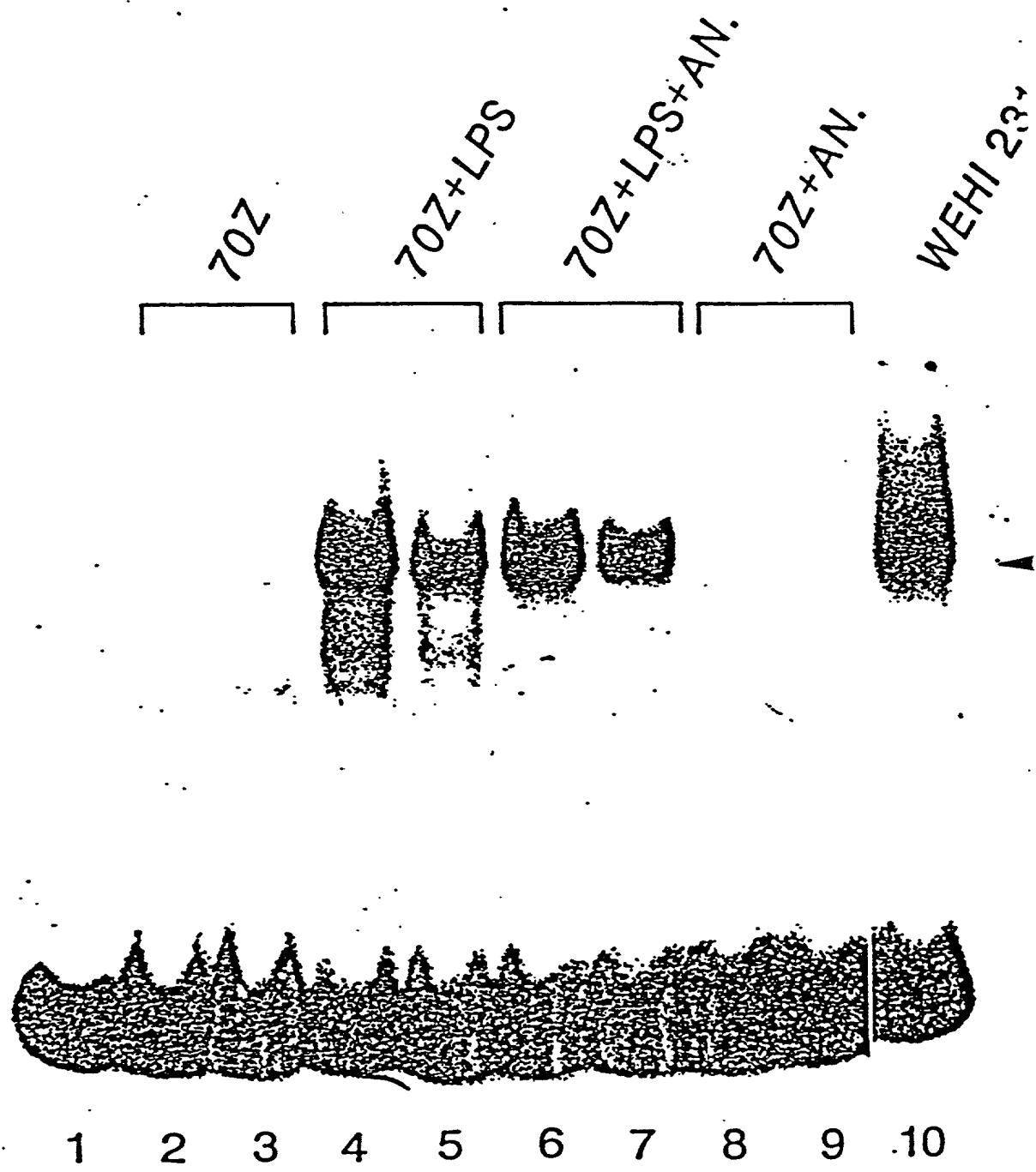


FIGURE 23A

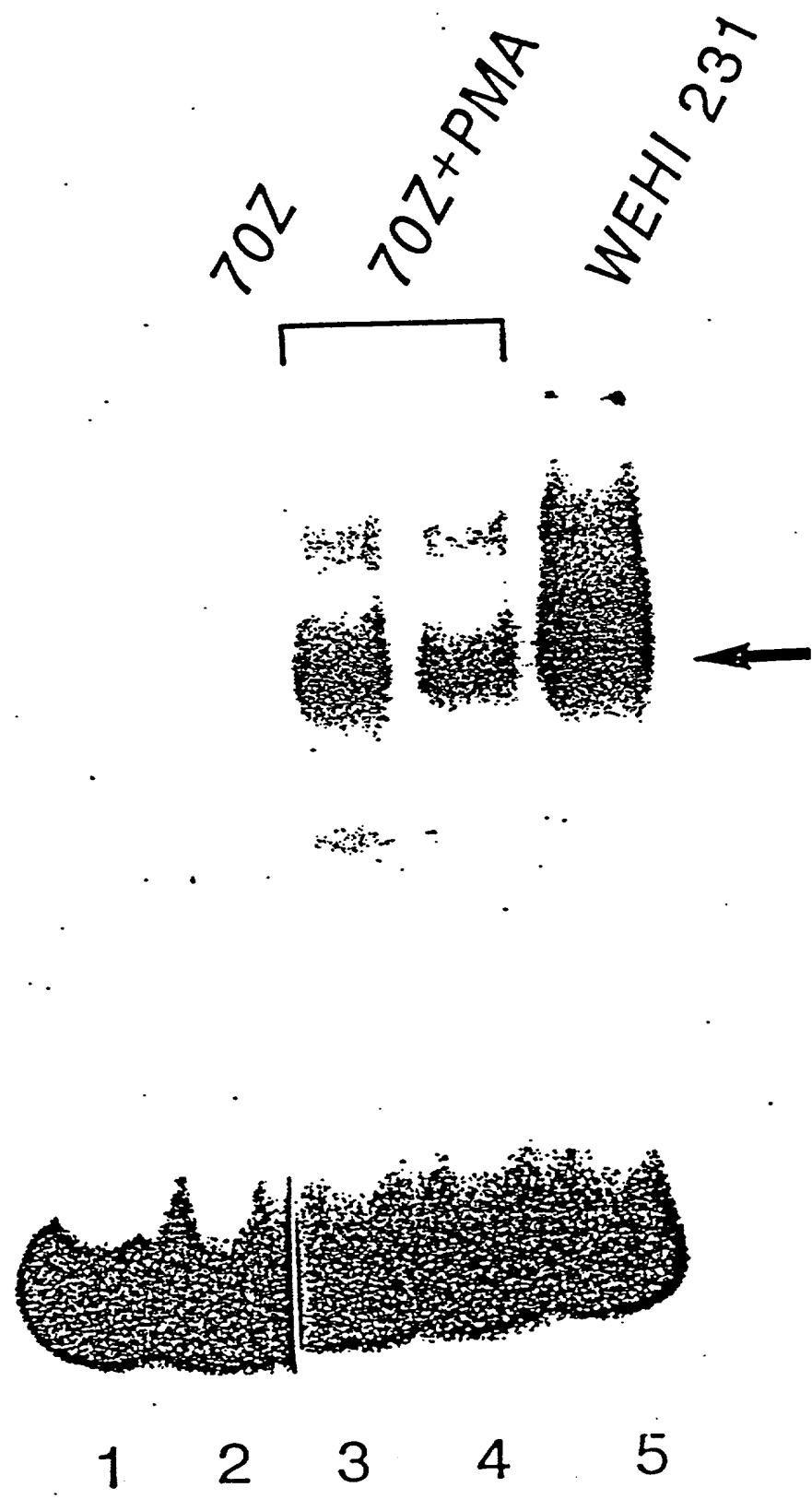


FIGURE 23B

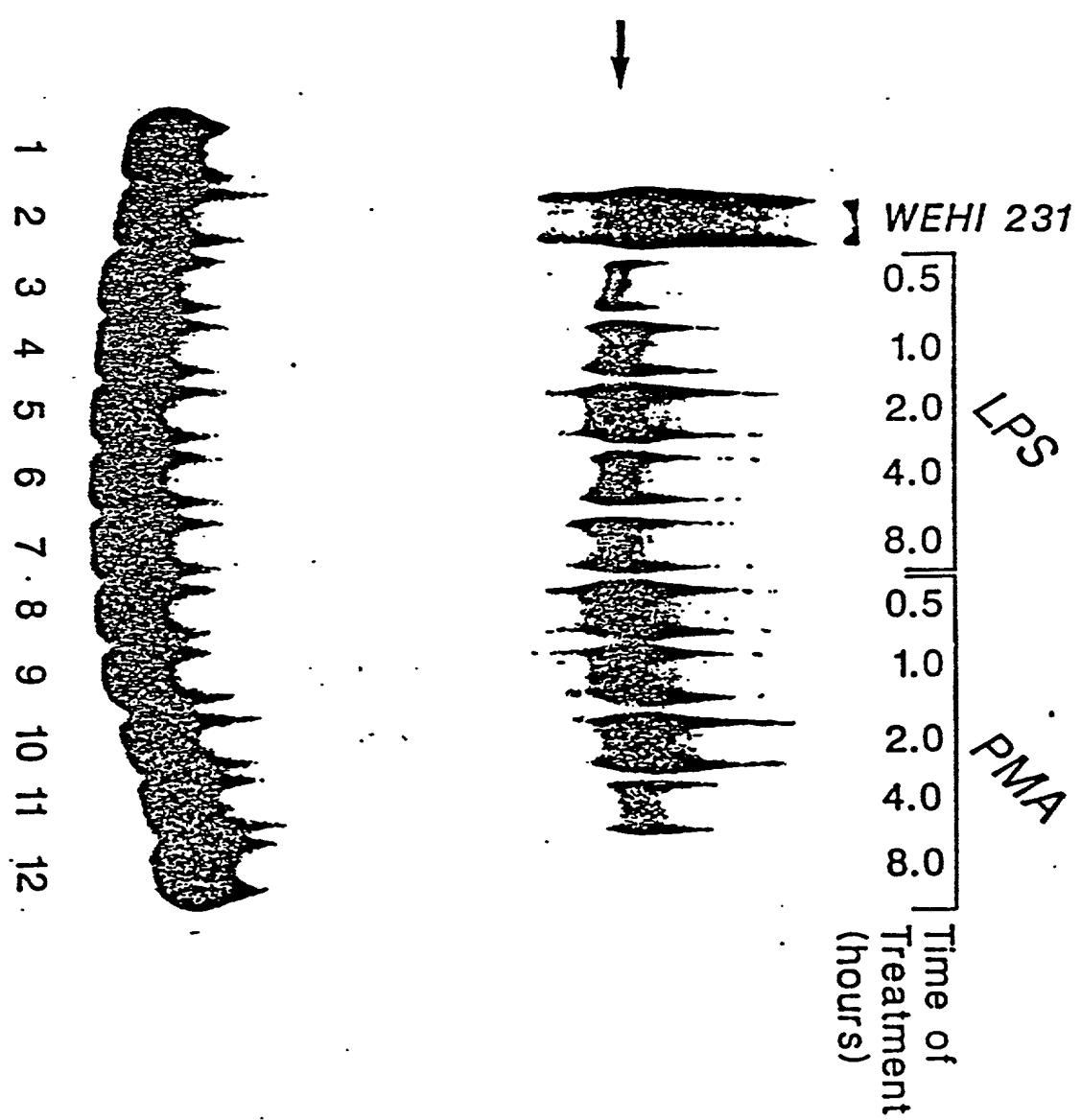


FIGURE 24A

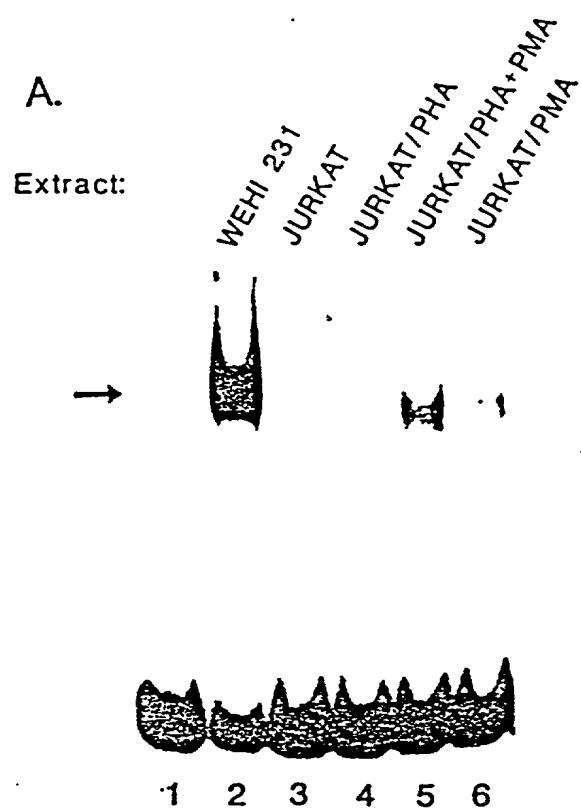


FIGURE 24B

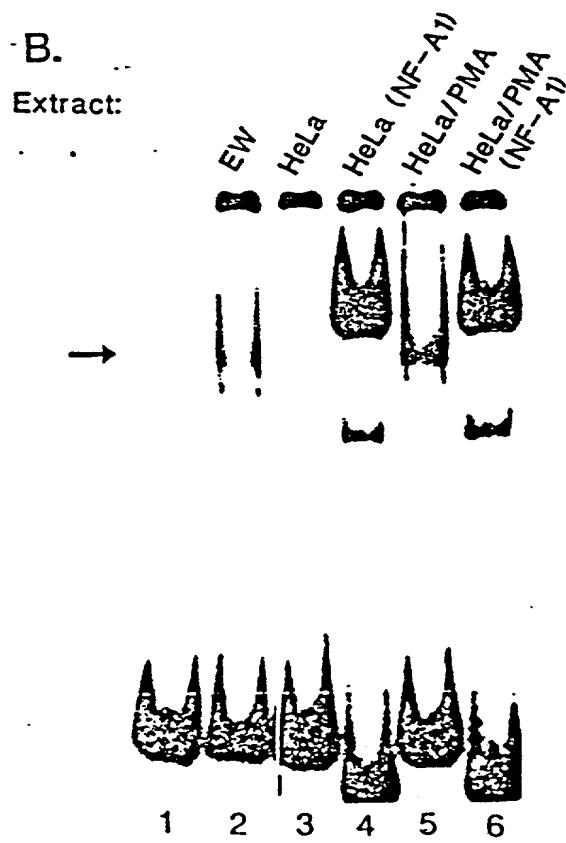


FIGURE 24C

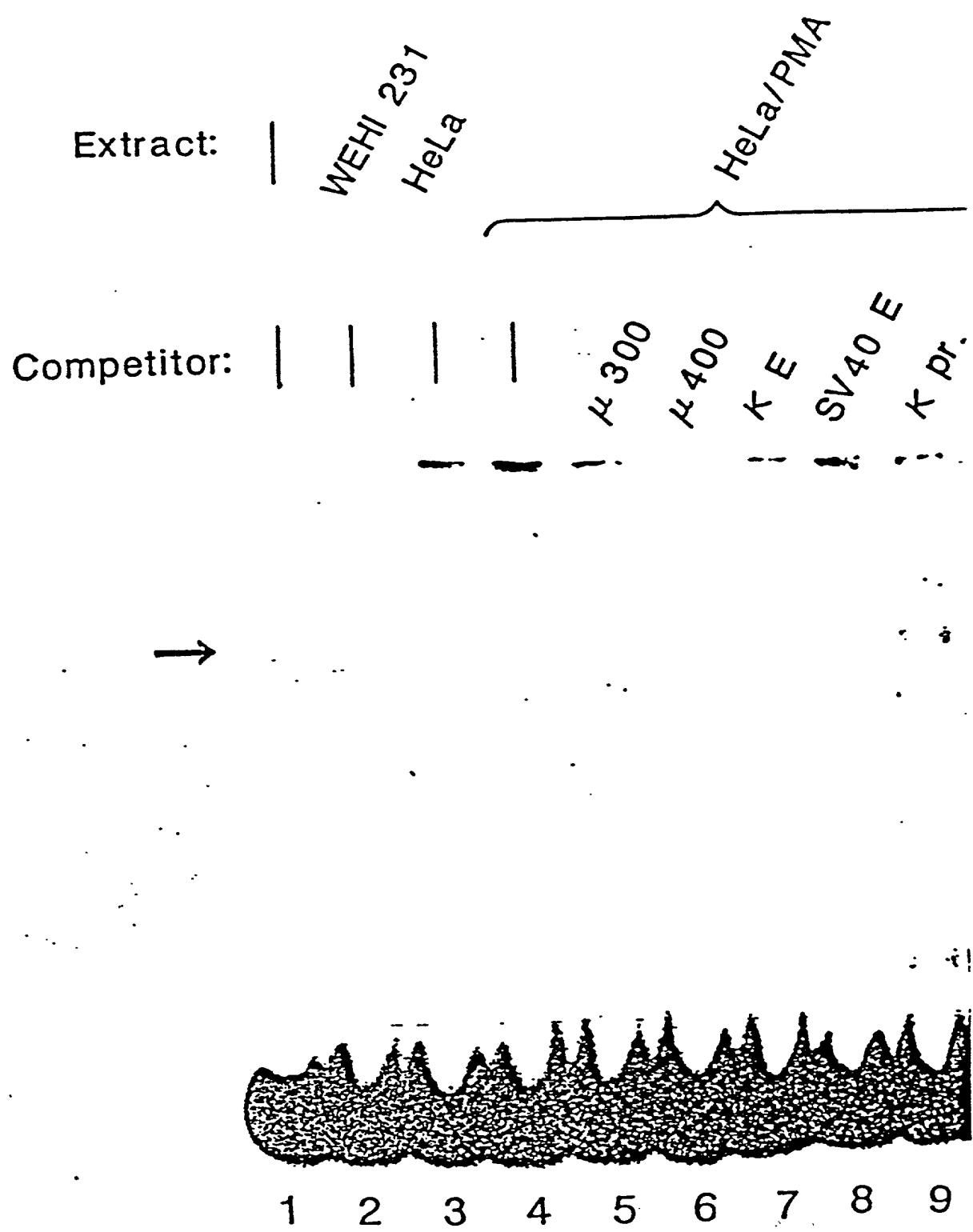
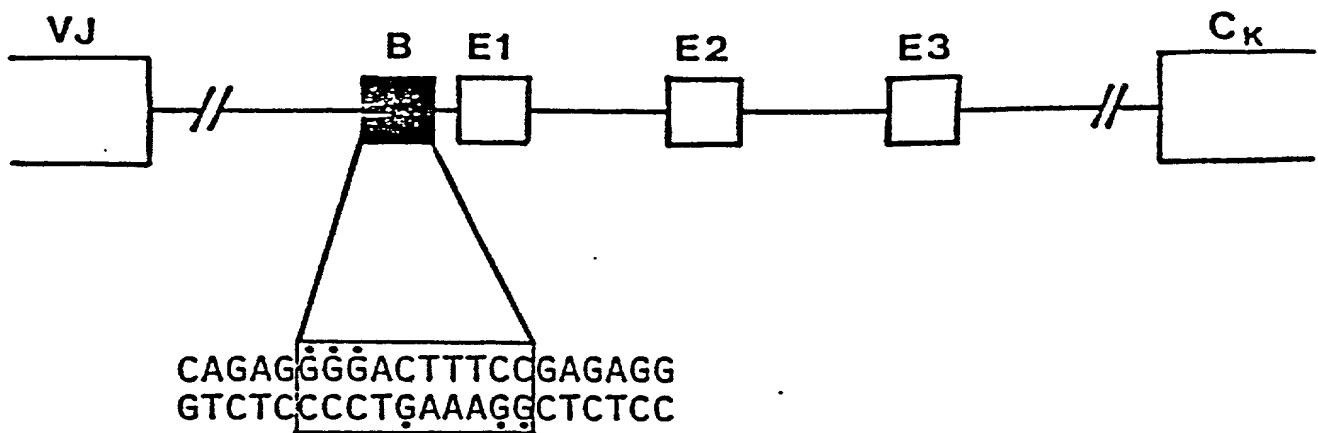


FIGURE 25

κ -Enhancer



HIV LTR

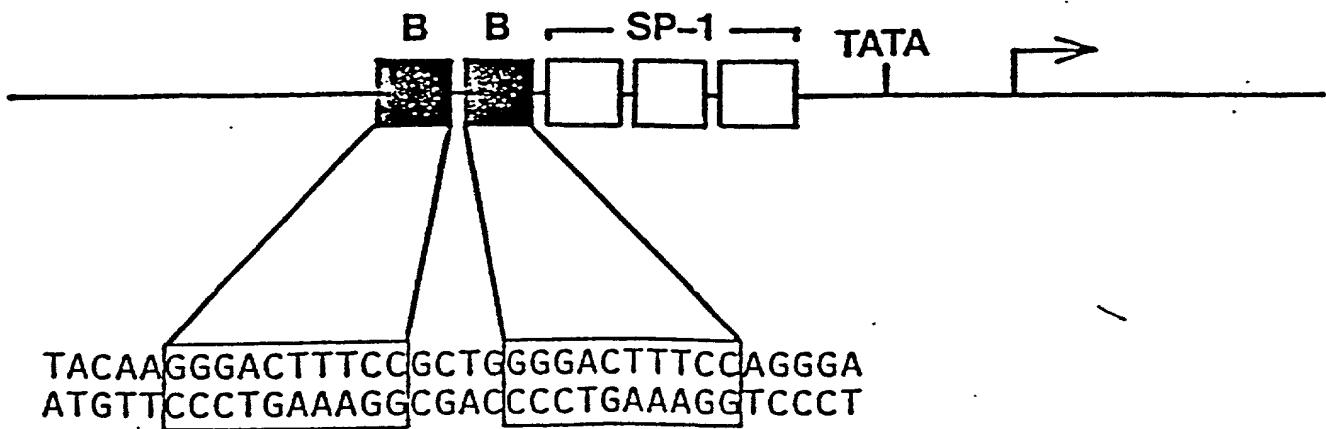
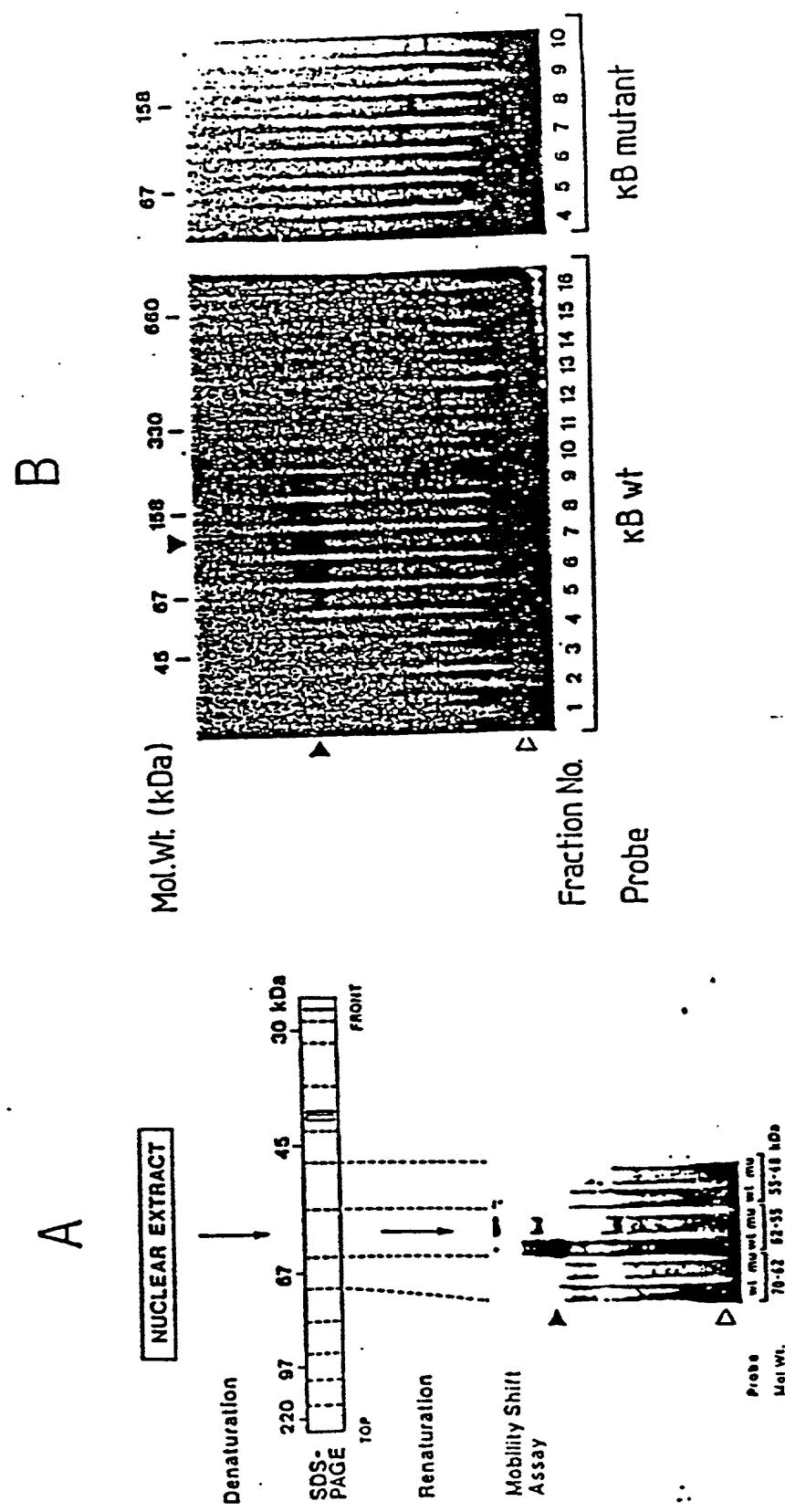


FIGURE 26



B

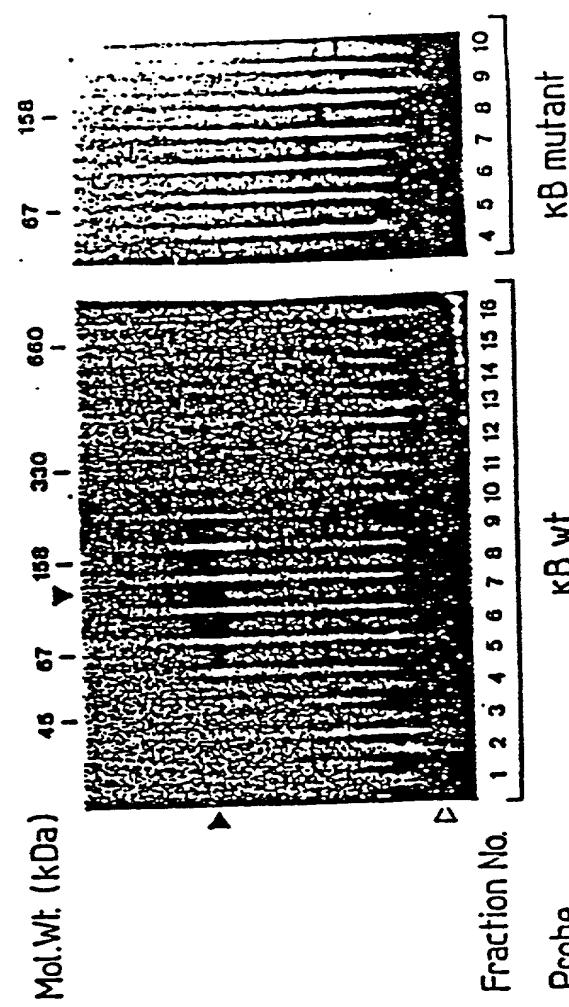


FIGURE 27. Effect of TPA on κ B nuclear factor.

A

B

C

Denaturation
SDS-PAGE
Renaturation

Dissociating
Agents

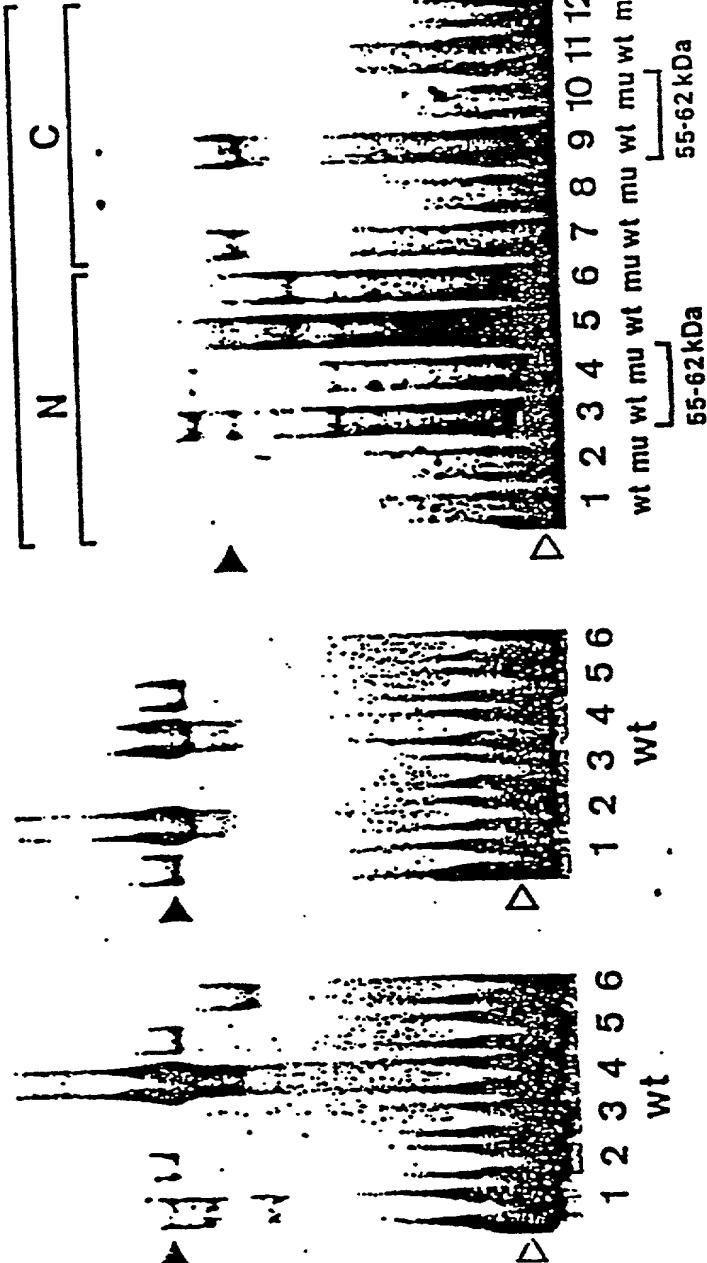
Treatment : none

CONTROL TPA

Fraction : N C P N C P

wt

CONTROL



κ B-Probe :

1 2 3 4 5 6 1 2 3 4 5 6 1 2 3 4 5 6 1 2 3 4 5 6

wt wt mu mu mu mu mu mu

55-62 kDa

FIGURE 28

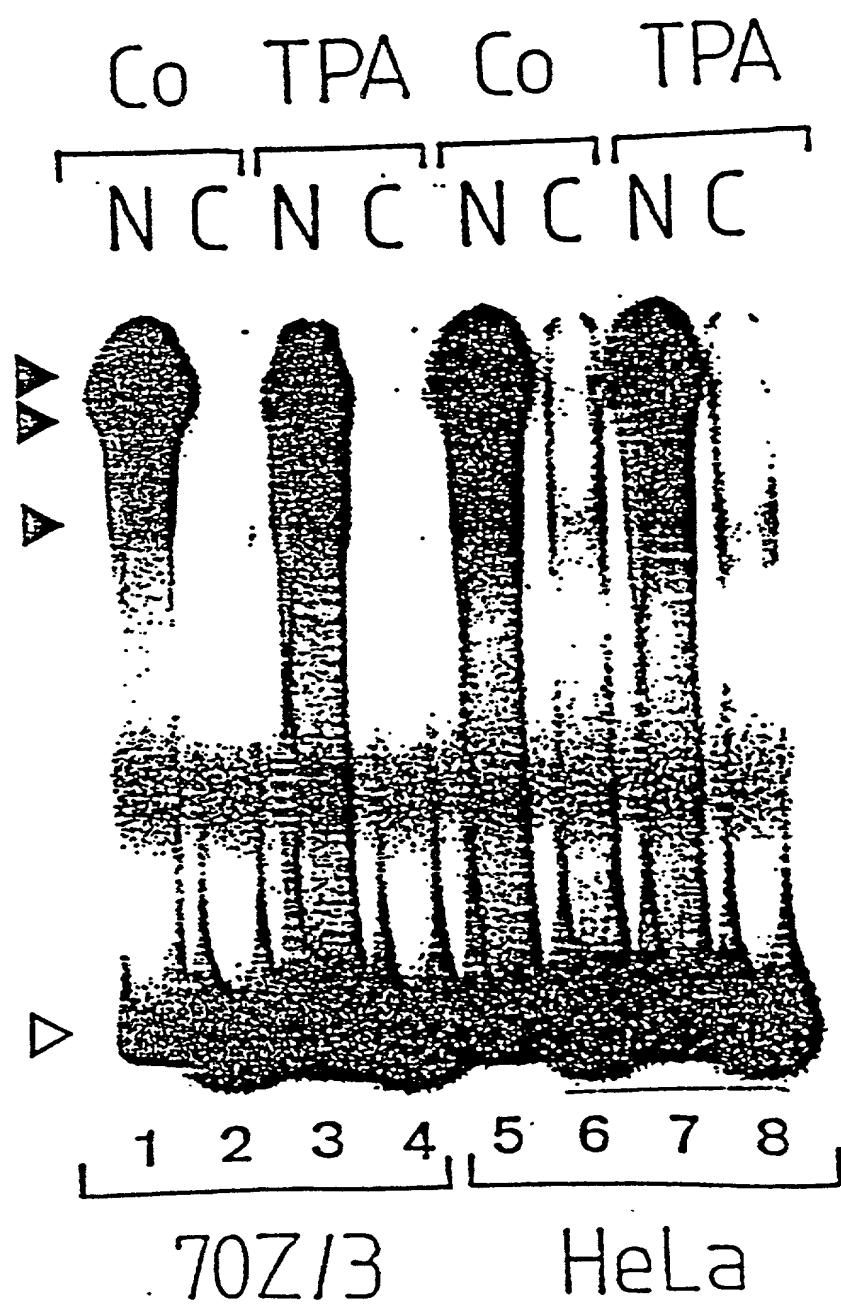


FIGURE 29

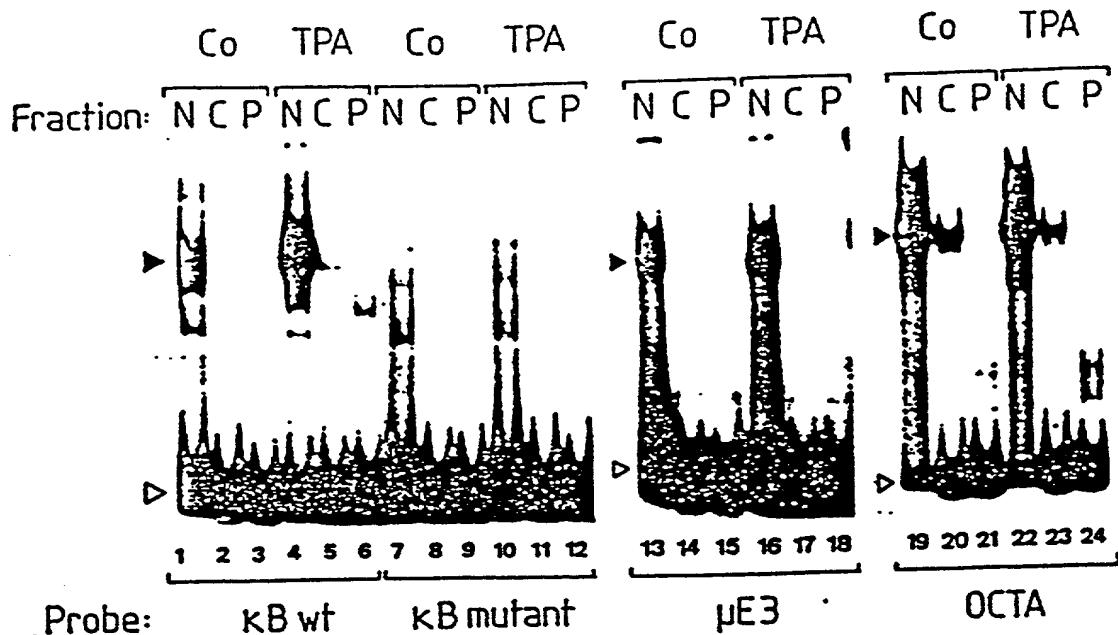


FIGURE 30

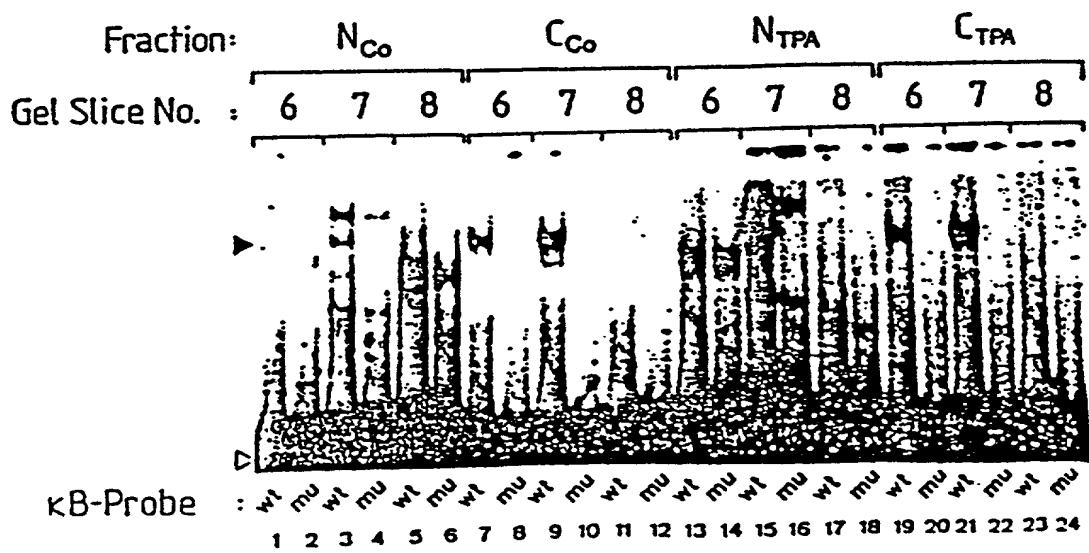


FIGURE 31

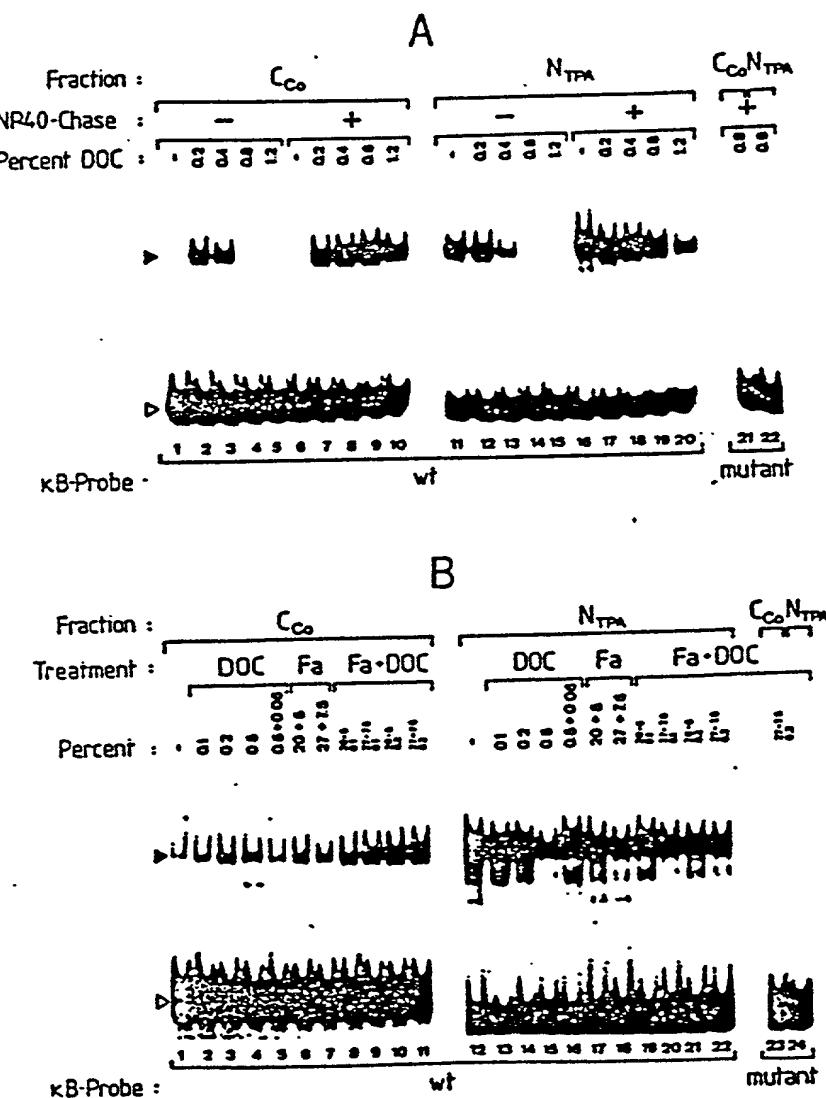


FIGURE 32

70Z/3

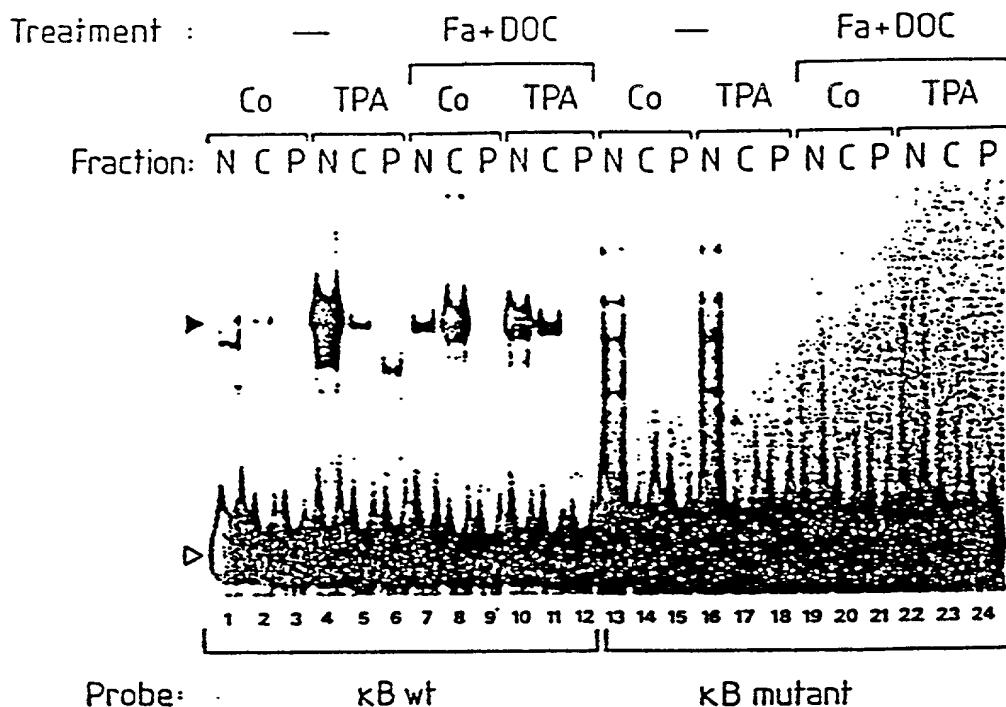


FIGURE 33

HeLa

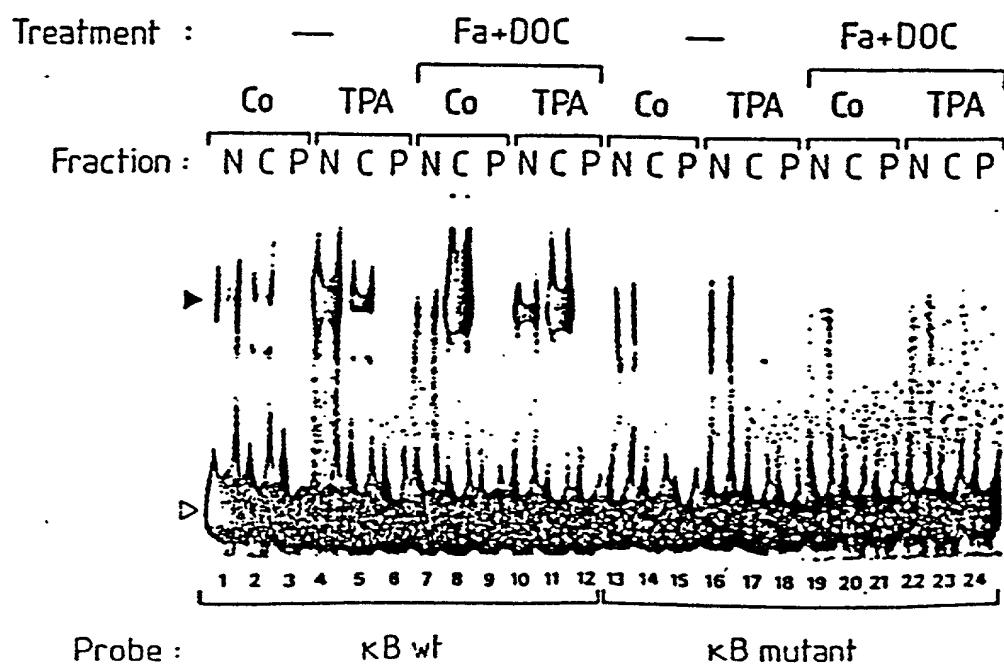
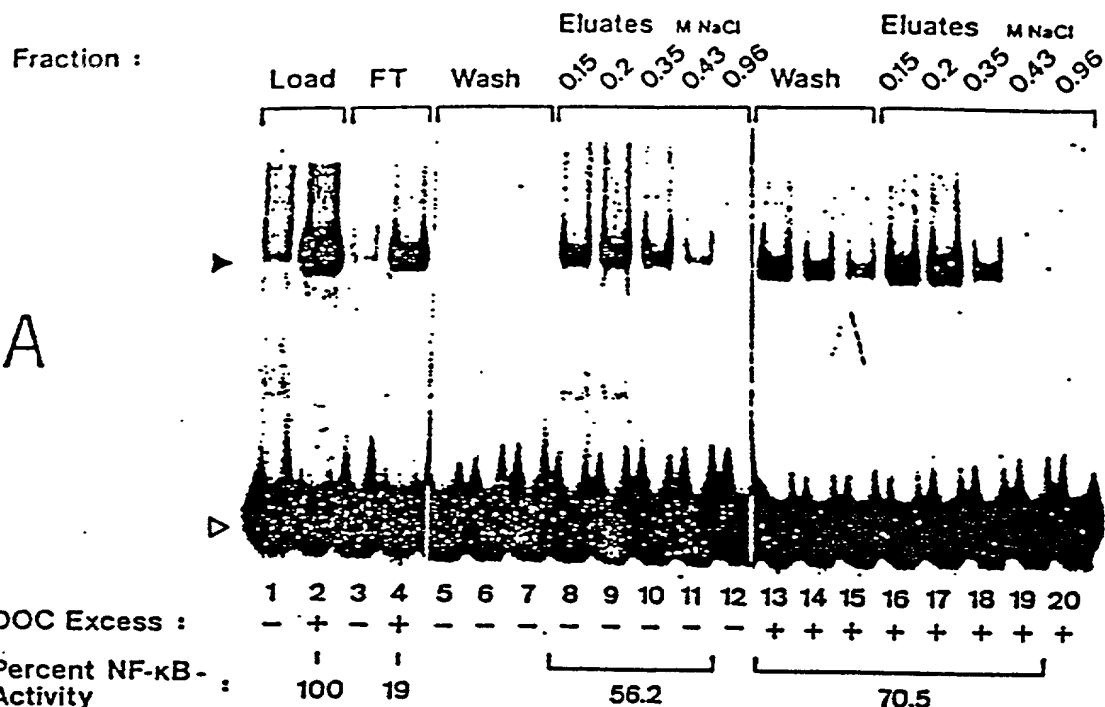


FIGURE 34



NF- κ B in		0.2M NaCl Fraction										Nuclear Extract (TPA)									
+Cytosol	:	4	-	-	1	2	4	-	-	-	-	4	-	-	1	2	4	-	-	-	-
+NF- κ B-depleted Cytosol	:	-	4	-	-	-	-	-	1	2	4	-	4	-	-	-	1	2	4

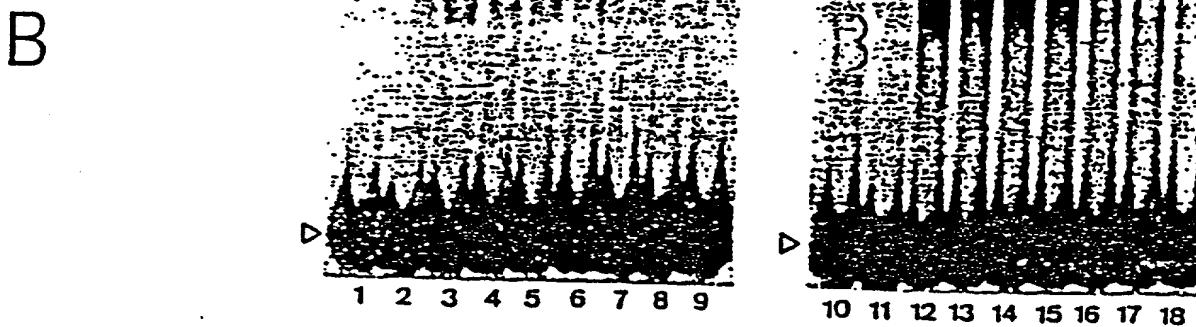


FIGURE 35

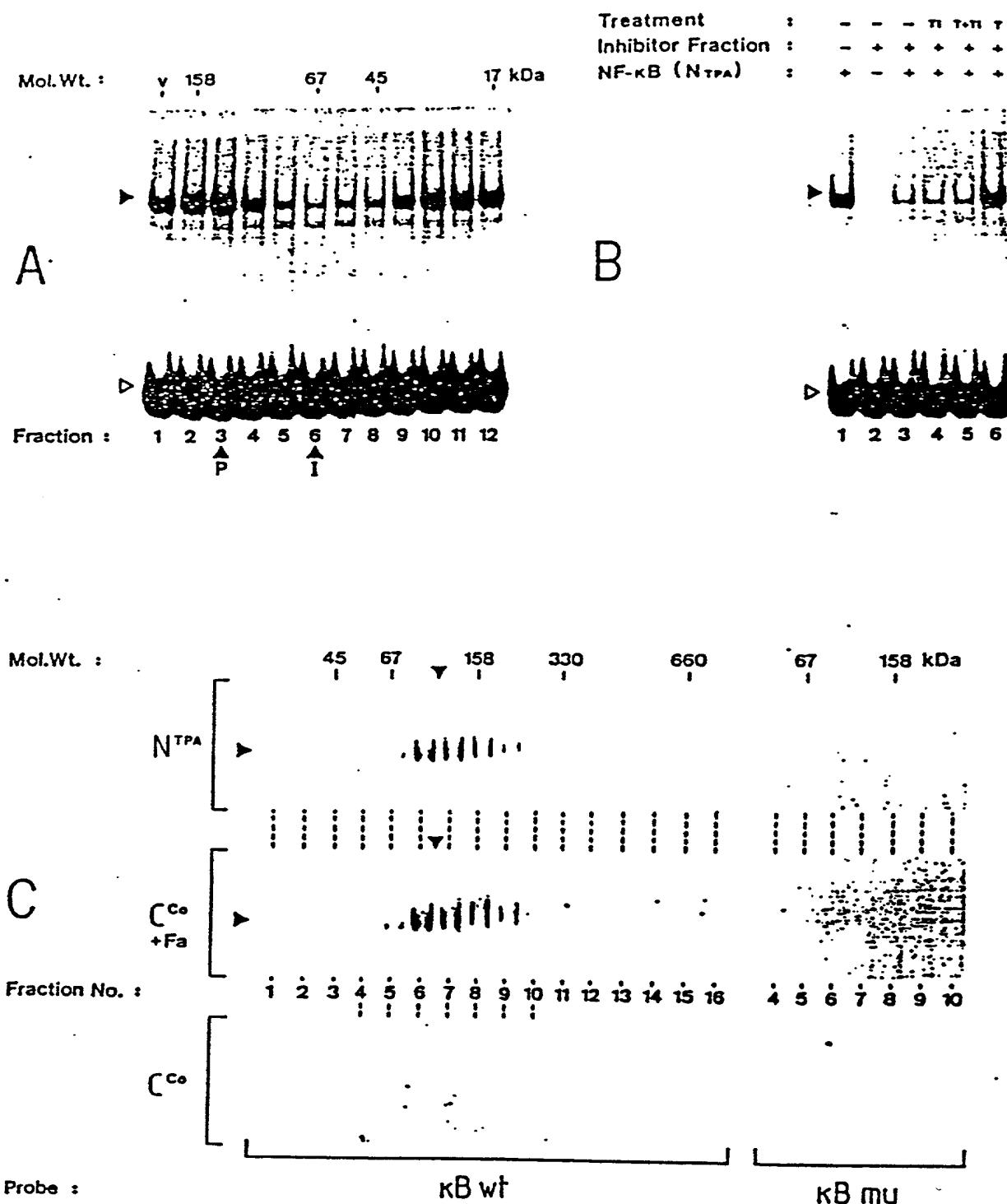


FIGURE 36

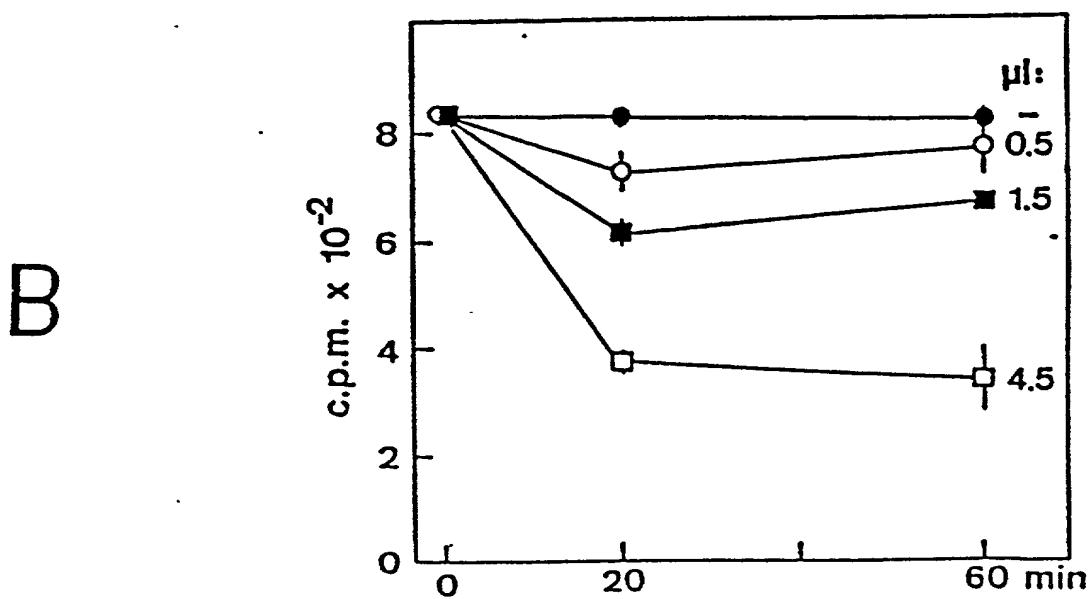
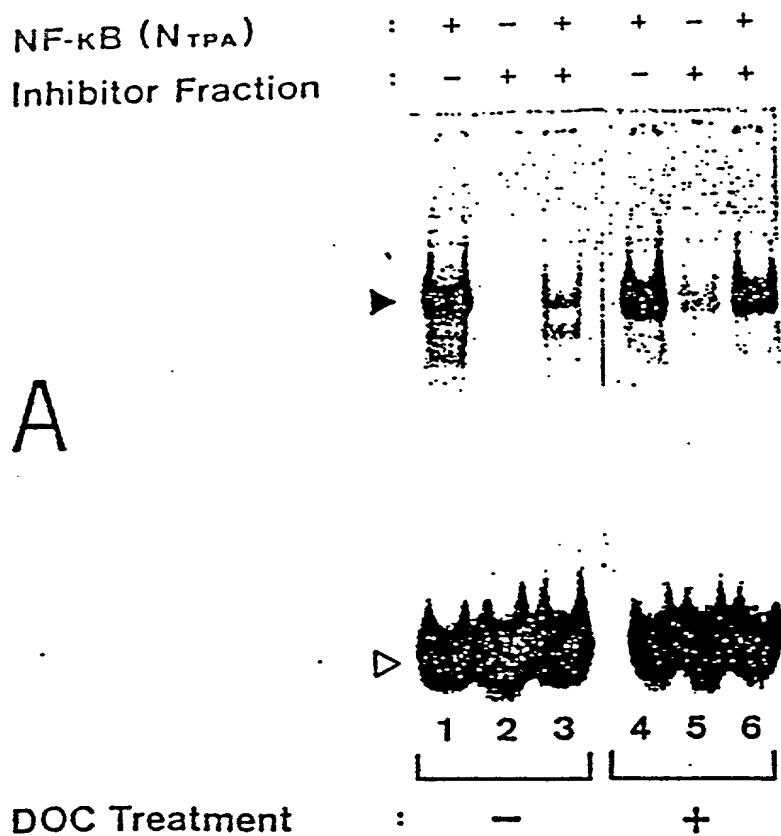


FIGURE 37

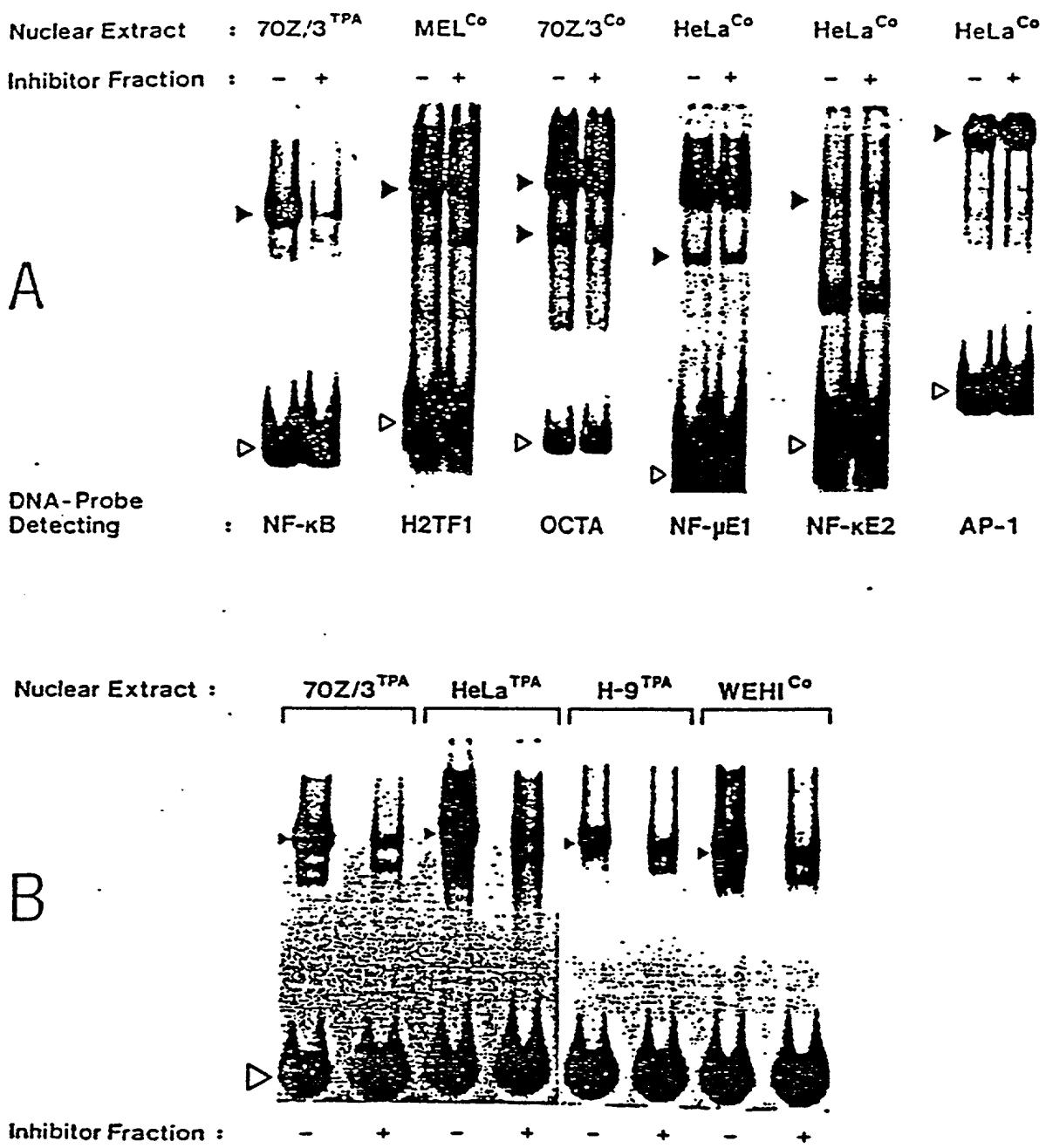
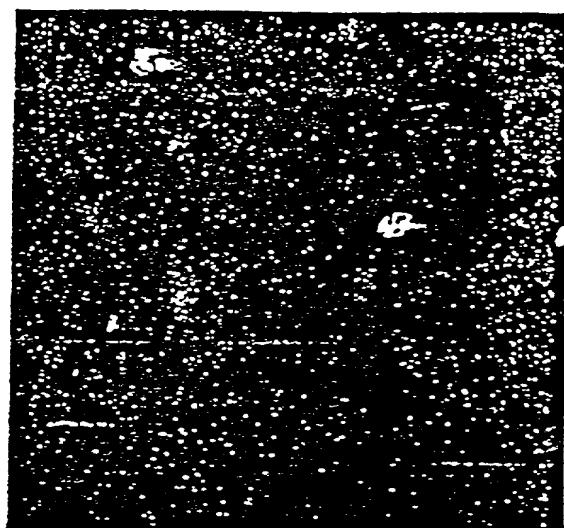


FIGURE 38

Phase Contrast



Nuclear Staining



A

Enucleation

: - +

Treatment of Cells

: Co TPA Co TPA

: - +

: Co TPA Co TPA

: - +

: Co TPA Co TPA

B



}



Probe

: 1 2 3 4

: kB

DOC-Treatment

: -

: 5 6 7 8

: kB

+

: 9 10 11 12

: AP-1

-

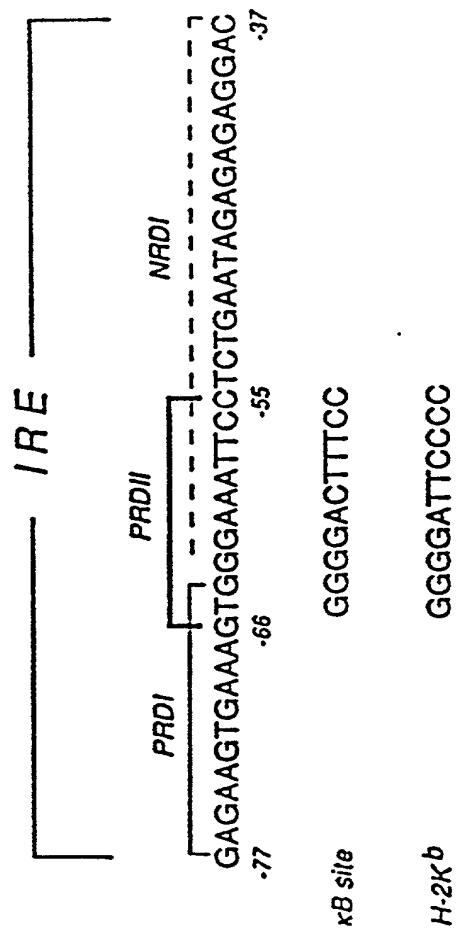


Figure 39

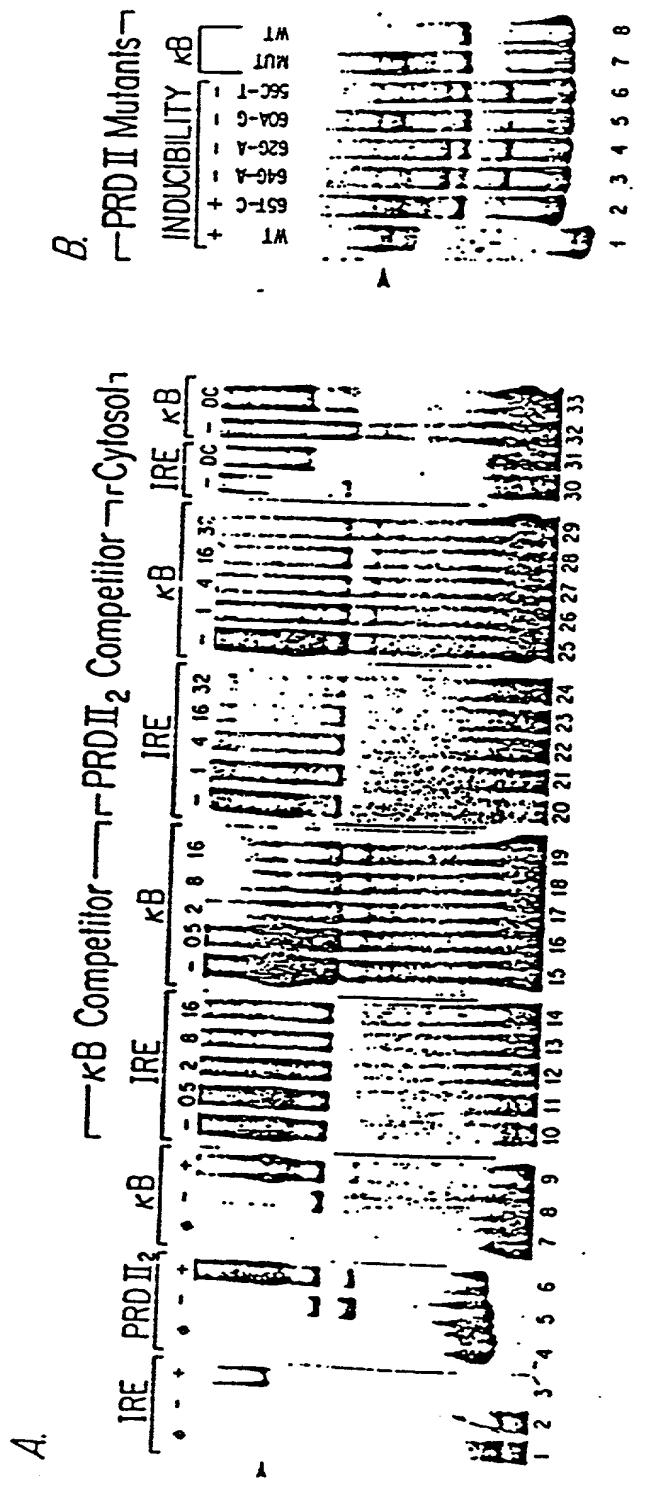


Figure 40

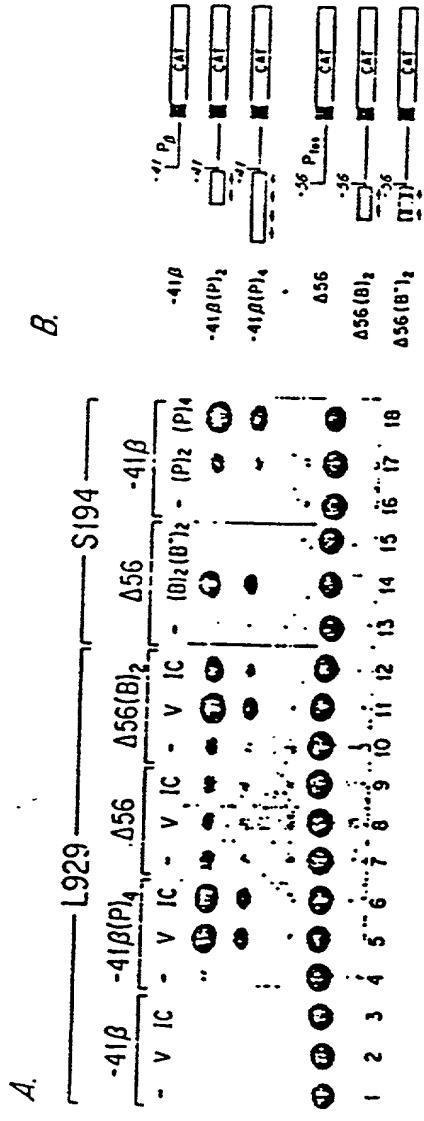


Figure 41

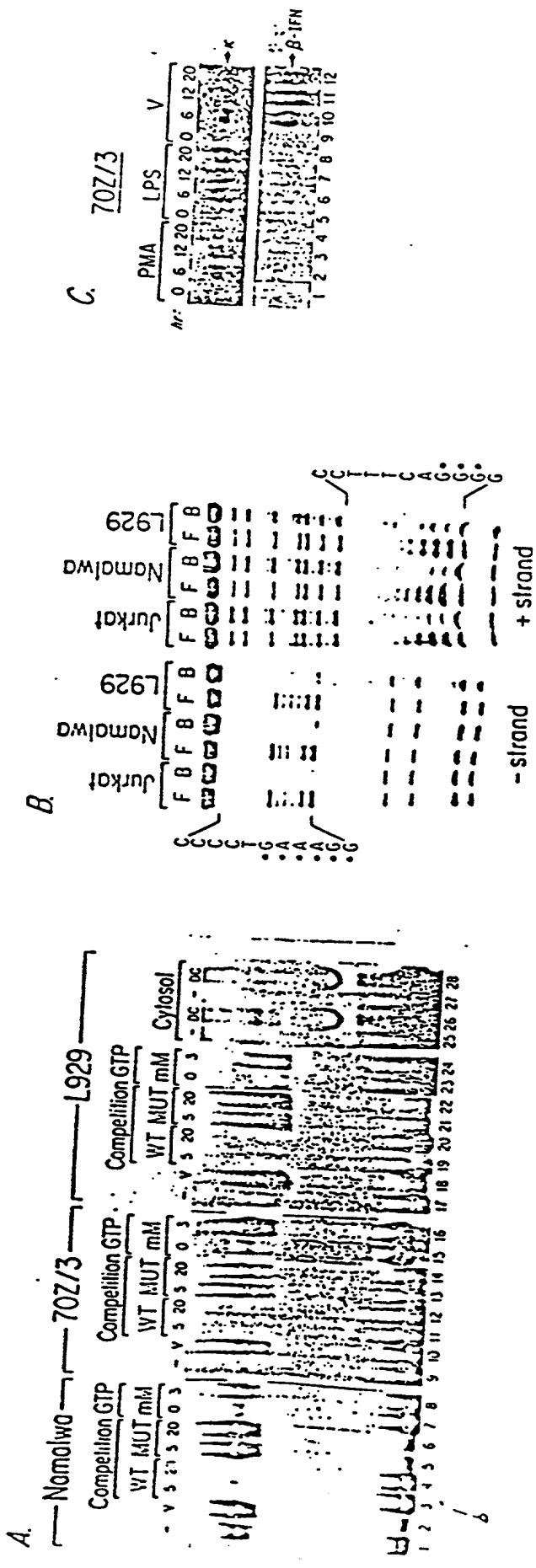


Figure 42

FIGURE 43